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Woodhead Publishing Series in Food Science, Technology and Nutrition:
Number 222

Handbook of food proteins

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Oxford Cambridge Philadelphia New Delhi

Published by Woodhead Publishing Limited,
80 High Street, Sawston, Cambridge CB22 3HJ, UK
www.woodheadpublishing.com

Woodhead Publishing, 1518 Walnut Street, Suite 1100, Philadelphia,
PA 19102-3406, USA

Woodhead Publishing India Private Limited, G-2, Vardaan House,
7/28 Ansari Road, Daryaganj, New Delhi – 110002, India
www.woodheadpublishingindia.com

First published 2011, Woodhead Publishing Limited
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British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Control Number: 2011934928

ISBN 978-1-84569-758-7 (print)
ISBN 978-0-85709-363-9 (online)
ISSN 2042-8049 Woodhead Publishing Series in Food Science, Technology and Nutrition (print)
ISSN 2042-8057 Woodhead Publishing Series in Food Science, Technology and Nutrition (online)

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Typeset by Toppan Best-set Premedia Limited, Hong Kong
Printed by TJI Digital, Padstow, Cornwall, UK

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Introduction to food proteins

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Abstract: This chapter provides a brief overview of the structural characteristics and functional properties of proteins that are used in food products. It highlights the mechanisms responsible for the key functional behaviour of both linear and globular proteins which are to form gels and to stabilise oil-in-water emulsions. It also notes the growing interest in the use of proteins as edible films to enhance the shelf life of fruit and vegetables and in biodegradable packaging. The chapter also introduces the scope of the book and the contributions provided by leading experts in this field of research covering a broad range of food proteins which are derived from various sources including, animal, botanical, macro-algal and micro-organisms.

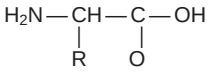
Key words: proteins, protein structure, gelatine, bovine serum albumin, beta lactoglobulin, milk proteins, egg proteins, protein gels, oil-in-water emulsions, interfacial behaviour, biodegradable packaging, edible films.

1.1 Introduction

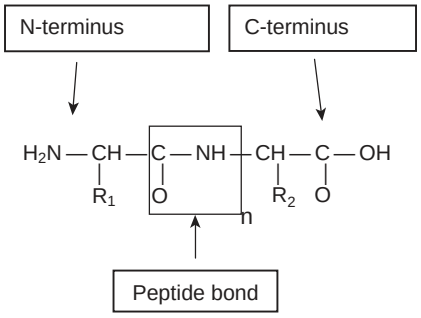
Proteins are present in all living things and have a key role in many biological processes such as cell signalling, cell adhesion and the immune response. They may also have a structural or mechanical function in, for example, the muscles and connective tissue of animals and the cell walls of plants. It is now recognised that proteins represent a valuable renewable resource and a number of proteins are processed on an industrial scale for application in a range of areas including food, cosmetics, pharmaceuticals, medicine, adhesives, packaging, coatings, etc. This book provides an overview of the source, structure, properties of commercially important food proteins and concentrates, in particular with their application as food additives and food ingredients. A list of common food proteins is presented in Table 1.1.

1.2 Structure of protein

There are 20 L-α -amino acids which are the building blocks of all proteins. Each amino acid contains a primary amine and carboxylic acid group with the general formula:



The R group differs for the various amino acids and can impart polar, non-polar, anionic or cationic characteristics. The amino acid units are linked together through a peptide bond to form a polypeptide chain of a characteristic length as illustrated below:



The free OH group at the C-terminus is available to form further peptide links and proteins consisting of 15–10,000 amino acids are known. Since proteins contain both cationic and anionic charges due to the presence of ionisable groups, notably amine and carboxyl, they have a characteristic isoelectric point which corresponds to the pH at which the molecules have a net zero charge.

The primary structure of proteins is defined by the characteristic sequence of amino acids of the polypeptide chain. Certain amino acids within the chain can give rise to local secondary structures such as the alpha helix and beta sheet and a variety of such secondary structures can exist within a single protein molecule. While some protein molecules adopt linear conformations, others fold to varying extents to form more globular structures and the overall shape of the protein, which is referred to as the tertiary structure, is stabilised by a range of interactions including hydrogen bonding, disulfide bonding and salt bridges. Hydrophobic amino acids tend to reside in the interior of globular proteins with hydrophilic amino acids at the periphery. Linear proteins function as structural elements, such as in the connective

Table 1.1 Source of common food proteins

Source	
Animal	Muscle proteins
	Blood proteins
	Proteins in the connective tissue
	Milk proteins
	Egg proteins
Botanical	Cereals
	Wheat, corn, barley, oats, rice
	Legumes and pulses
	Peas, soybeans, lupins, lentils
	Tubers
	Potato
	Oil seeds
	Rapeseed, cottonseed, peanut
Macro-algae	Green and blue-green seaweed
	Spirulina, Anabaena, Nostoc, Ulva, Enteromorpha
Micro-organisms	Fungi
	Mycoprotein

tissue of animals. The polypeptide chains are arranged in parallel forming long fibres; examples include collagen found in tendons, cartilage and bone, and keratin in hair, skin and nails. Globular proteins are usually soluble in an aqueous environment and are involved, for example, in transport processes or dynamic functions in the cell.

1.3 Functional properties of proteins

Whilst it is clear that proteins have a major function in many biological processes, they also have a key role as food additives and ingredients. As will become clear on reading the various chapters in this book, a common feature is the ability of many proteins to form gels, to stabilise emulsions and foams and to form films.

1.3.1 Gelation

Globular proteins will normally unfold on heating and at sufficiently high concentrations the denatured chains will aggregate to form thermally irreversible gels. The properties of the gels will depend on a number of factors including the degree of unfolding of the protein chains and the extent and kinetics of chain aggregation. Denaturation can also occur at extremes of solution pH and ionic strength. Linear association of the denatured molecules leads to the formation of uniform finely stranded three-dimensional

network structures. Association is likely to be driven by interaction between hydrophobic domains along the protein chain which become exposed when the molecules unfold. When the electrostatic charge on the protein chains is significantly reduced, micron-sized protein aggregates are formed, which then associate to form coarse network structures. Transmission electron micrographs of a range of heat-set globular protein gels are presented in Fig. 1.1. The micrographs show the variations in microstructure that can be obtained for different proteins under different solution conditions.

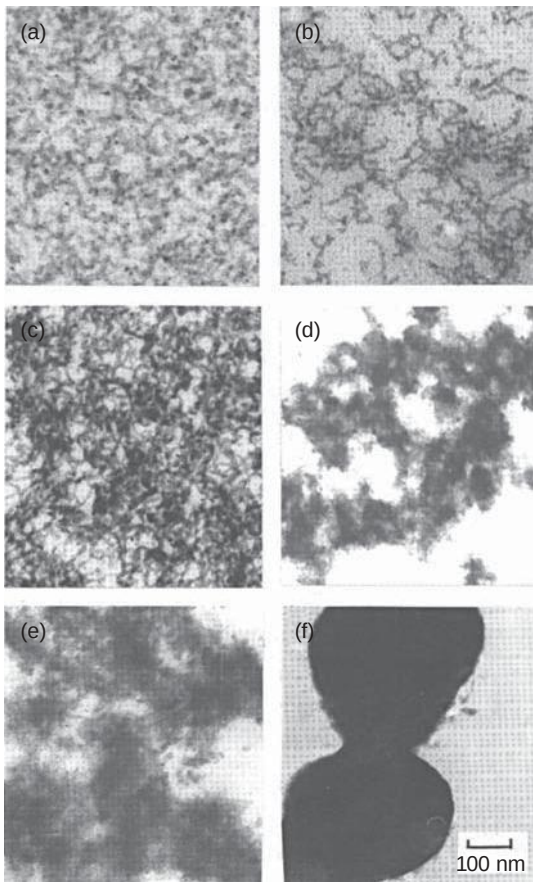


Fig. 1.1 Transmission electron micrographs of: (a) 10% beta lactoglobulin at pH 7; (b) 15% soy glycinin at pH 3; (c) 10% alpha lactalbumin at pH 7; (d) 15% alpha chymotrysin at pH 3; (e) 10% alpha lactalbumin pH 7, 100 mM NaCl; (f) 10% BSA coagulate pH 5.1 [reproduced from A.H. Clark 'Gelation of globular proteins' in *Functional properties of food macromolecules* S.E. Hill, D.A. Ledward and J.R. Mitchell eds Aspen Publishers Inc. Maryland USA 1998 p 77].

In contrast to globular proteins, gelatine, which is a fibrous protein derived from collagen, is able to form thermally reversible gels. Collagen is an extracellular protein present in the bones, skin and connective tissue of humans, animals and fish and adopts a triple helical conformation in the native state. However, in the production of gelatine, the collagenous materials are treated with acid or alkali and the collagen molecules denature producing the heterogeneous material referred to as gelatine. In solution the gelatine molecules adopt a disordered conformation at high temperatures but on cooling to approximately 25°C they undergo a thermally reversible coil-helix transition and the molecules partially reform the collagen triple helical structure. The stiff helical chains will then self-associate to form a three-dimensional gel structure. The degree of helical content and overall rheological properties will be dependent on the solvent conditions and on the rate of cooling. When the gel is re-heated, the association is disrupted and the molecules adopt a disordered structure and the gel melts. Gel melting is usually observed at approximately 37°C and the reason that the melting temperature is greater than the setting temperature is that the helices must disaggregate before the helix-coil transition can occur.

1.3.2 Interfacial properties

Proteins, particularly those derived from milk and eggs, are commonly used to stabilise oil-in-water emulsions and foams because they are able to adsorb at the oil-water and air-water interfaces. Figure 1.2 shows transmission electron micrographs of various emulsions stabilised by casein. Figure 1.2(a) shows a thin layer of sodium caseinate at the interface of soya oil emulsion droplets and Figs 1.2(b) and 1.2(c) show the attachment of micellar casein (dark areas) at the oil-water interface for homogenised milk samples. The surface activity of different proteins will be a function of their molecular size and conformation. The amino acid composition will control the overall amphiphilic characteristics and the protein's ability to adsorb at interfaces. During emulsification the role of the protein is to adsorb onto the newly created surface of the oil droplets and prevent droplet aggregation and coalescence. To this end smaller protein molecules are expected to be more effective, since they are able to diffuse to the surface at a faster rate. Larger protein molecules, however, are likely to provide more points of contact and increase the overall energy of adsorption. For globular proteins the ability to unfold at the interface and expose the hydrophobic amino acid groups to facilitate adsorption is a key factor. In addition solvent quality will be important and it is expected that adsorption would be greatest under poor solvent conditions at pH values close to the isoelectric point. Figure 1.3 shows the adsorption isotherms for egg white protein adsorbing onto limonene at pH 3.5 and 7.5. It is noted from the adsorption plateau that the maximum amount adsorbed (corresponding to complete coverage of the droplet surface) is ~1–1.5 mg m^{-2} and also that from the initial slope

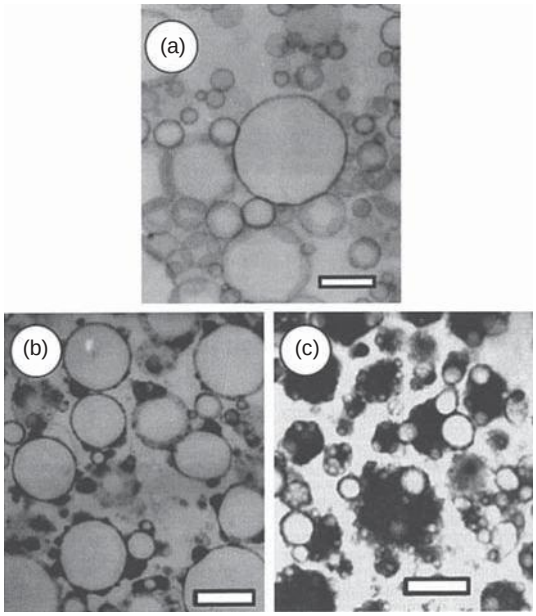


Fig. 1.2 Transmission electron micrographs of (a) a soya oil emulsion stabilised by sodium caseinate, (b) and (c) micellar casein adsorbing at the interface of fat globules in homogenised milk [from D.G. Dalgleish 'Food emulsions – their structures and structure forming properties' *Food Hydrocolloids* 20 415–422 (2006)].

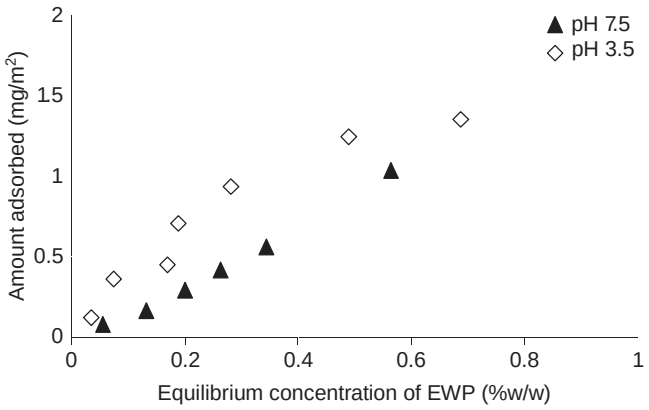


Fig. 1.3 Adsorption of egg white protein onto limonene oil droplets at pH 3.5 and 7.5 [adapted from S.R. Padala, P.A. Williams and G.O. Phillips 'Adsorption of gum Arabic, egg white protein and their mixtures at the oil-water interface in limonene oil-in-water emulsions' *J. Agricultural and Food Chemistry* 57 4969–4973 (2009)].

of the isotherm that the adsorption is low affinity. The difference in the amount adsorbed at the two pHs can be attributed to differences in the net electrostatic charges on the proteins and/or differences in protein conformation. These observations are typical for protein adsorption generally.

Once adsorbed at an interface, proteins can self-associate to form elastic networks as has been confirmed by surface rheology measurements. A clear illustration of the network structure has also been provided by atomic force measurements (AFM) as in the case of beta casein adsorbed at the air-water interface in a Langmuir Trough. AFM images of the protein at the interface on addition of a surfactant are presented in Fig. 1.4. The surfactant is able to displace the adsorbed protein molecules over time, and dark areas, which are devoid of protein, appear. The protein molecules which remain at the interface clearly show network connectivity.

Proteins are able to stabilise oil droplets against flocculation and coalescence since the adsorbed protein layers can give rise to electrostatic repulsive forces at pH values away from the isoelectric point where they will carry a net positive or negative charge. At pH values close to the isoelectric point, where electrostatic repulsive forces are not sufficient to prevent aggregation, stabilisation can be achieved through steric repulsive forces arising from the enthalpic and entropic interactions between the adsorbed protein layers. However, in many cases the adsorbed protein layer is too thin to provide steric stabilisation and there is considerable current interest in using combinations of proteins and polysaccharides. One approach is to produce an emulsion initially with a protein as the emulsifier and add a polysaccharide which then interacts with the adsorbed protein forming a bilayer. The bilayer has enhanced charge and increased thickness thus giving increased stability against droplet aggregation. An alternative procedure being explored is to form polysaccharide-protein electrostatic complexes (either in soluble form or in the form of a coacervate) and then use the complex as the emulsifier. Figure 1.5 shows the droplet size for emulsions prepared using sodium caseinate and dextran sulphate. In one set of experiments the sodium caseinate was adsorbed first and then varying concentrations of dextran sulphate added to form a bilayer. In the other

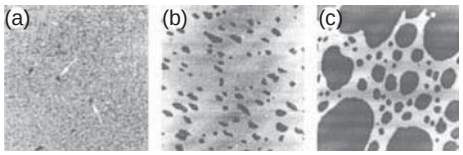


Fig. 1.4 AFM images of beta lactoglobulin adsorbed at the air-water interface [from N.C. Woodward, A.P. Gunning, P.J. Wilde, B-S. Chu and V.J. Morris, 'Engineering interfacial structures to moderate satiety' in *Gums and Stabilisers for the Food Industry* 15 P.A. Williams and G.O. Phillips eds Royal Society of Chemistry Publishers, Cambridge, UK (2010) p 367].

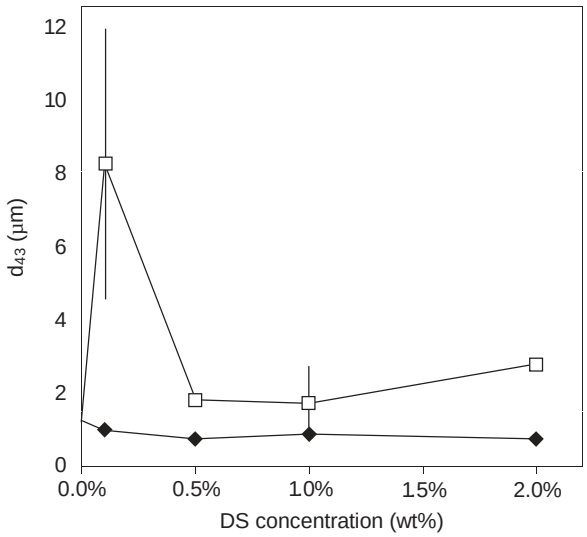


Fig. 1.5 Droplet size of emulsions prepared using sodium caseinate and varying concentrations of dextran sulphate. The squares represent emulsions prepared by adsorbing the caseinate first and then adding the dextran sulphate, while the diamonds represent emulsions prepared using soluble sodium caseinate – dextran sulphate electrostatic complexes [from L. Jourdain, M.E. Leser, C. Schmitt, M. Michel and E. Dickinson *Food Hydrocolloids* 22 647–659 (2008)].

experiment soluble sodium caseinate–dextran sulphate electrostatic complexes were used. It was found that the soluble complexes were more effective based on the fact that the emulsions produced had a smaller droplet size. There has also been considerable interest in recent years in forming polysaccharide–protein Maillard conjugates in which the polysaccharide and protein are covalently linked. This is achieved through the interaction between the reducing end of a polysaccharide and a primary amine group on the protein. In this process the polysaccharide and protein are dry blended and left at an appropriate temperature and relative humidity for the reaction to occur. Most of the studies reported have involved complexation of protein with non-ionic polysaccharides such as dextran, galactomannan and maltodextrin. The emulsification properties have been shown to improve with increasing molecular mass of the polysaccharide.

1.3.3 Film formation

There has been considerable interest in recent years in the application of proteins such as gelatine, whey, soy, corn zein and wheat gluten, for use in biodegradable packaging and as edible films to enhance the shelf life of fruit and vegetables. Edible coatings, when applied to fruit and vegetables,

can provide a barrier to moisture and carbon dioxide–oxygen exchange, and also improve mechanical handling properties. There is considerable interest at present in the development of 'active packaging' for food products which facilitate increased shelf life of food products. Protein films have been produced which incorporate various bioactive compounds including essential oils (oregano, pimiento, garlic, lemongrass), organic acids (sorbic, acetic, proprionic acid), bacteriocins (nisin) and enzymes (lysozyme). The bioactive compounds are released either by the film swelling in the presence of fluid from the food or by the protein film degrading. The rate of release of the active compound can be controlled by crosslinking the protein molecules with, for example, glyoxal, calcium ions or transglutaminase depending on the protein used.

1.4 Scope of this book

This volume contains chapters that deal in more detail with the structure, properties and applications of individual food proteins from a variety of sources. The initial chapters by B.T. O'Kennedy and M. Boland are concerned with milk proteins. Milk has traditionally been a major source of good nutritional protein and offers an array of specialised casein and whey-based components for specific applications. The liquid nature of milk allows effective fractionation of these components. The caseins are the main protein group in bovine milk. B.T. O'Kennedy, in a comprehensive chapter, describes the various products which can be produced, starting from skimmed milk which contains about 35% protein of which the caseins account for 80%. As noted in the section above, the applications of caseins are many and varied and the structural and technical bases for these are authoritatively described.

Whey proteins are another by-product from the dairy industry being used as concentrates containing up to 90% protein, which can enrich the nutritional value of infant food and provide valuable amino acids, essential for muscle development. The source, processing, chemistry and applications are fully dealt with in the chapter by Mike Boland. Increasingly these proteins are being used to stabilise foams and emulsions and in combining these functions can produce novel structures in cake toppings, for example.

There is a natural and important link between the collagen-derived gelatin and the array of products which can be derived directly from meat as described in the chapter by Rodrigo Tarté. There is some overlap with the gelatin chapter but taken together they cover meat tissues, connective tissues and blood. These, for many of us, provide the tasty goodness in our food. The regulator is strict about what can be termed "meat" specifying maximum fat and connective tissue contents for such designated ingredients. The chapter covers how these products can be obtained, their functional properties, food applications and current regulatory aspects for each.

Haug and Draget review the ubiquitous gelatin, which despite the controversy associated with it, continues to be a major food textural ingredient with more than 300,000 tons used annually. Mammalian, poultry and fish-derived collagen are described. The danger of disease transmission from the animal source has given rise to extensive regulatory monitoring procedures and its gel-forming properties provide an important characterisation parameter. Both chemically modified gelatin and hydrolysed products lead the way to an imposing range of applications in confectionery, foods, pharmaceuticals, medical products, cosmetics and photographic films. Whatever the problems the product has faced, it has weathered the storm and moved ahead into interesting and important new areas such as human nutrition.

Seafood proteins are expertly dealt with by Reza Tahergorabi, Seyed Vail Hosseini and Jacek Jaczynski. The authors start with the stark but accurate fact – *Seafood is the only source of animal protein that is still provided in significant amounts to human diet through capture of wild species*. They then proceed to outline its important biological value and as a source of essential amino acids. They consider also the long-term future of the industry and point to the new technologies needed to derive full benefit from this valuable and tasty source of protein. The treatment of the chemistry of these proteins is fascinating. It is a comprehensive and valuable treatment of a very diverse subject, making the information accessible at several levels.

The contribution on egg proteins is made by Ulrich Kulozik and Thomas Strixner who immediately and dramatically tell us how important eggs are to health and well-being: *the primary aim of the laying hen is not to produce high-value human food but to give rise to new life. Therefore, avian eggs contain the basic elements for life, and many of the egg compounds have so-called biological activity*. So are egg products the original functional food? The presentation is divided into several parts. First information is presented about the structure, composition, extraction and properties of egg yolk and this is followed by a review of the current understanding of egg yolk properties, interactions between constituents and possible applications due to different manufacturing steps. Subsequent parts describe the chemical characteristics and application of egg white components. The regulatory position of egg proteins as food allergens is clarified. No wonder mother insisted on an egg for breakfast every day!

After a slow start it is evident that soy proteins have taken off as a health food, particularly in the USA following FDA approval that 25 grams of soy protein a day may reduce the risk of heart disease. The chapter by D. Fukushima deals with the structures and nutritive value of the soy proteins including the genetic improvements that have been achieved.

Susan Arntfield is reponsible for two excellent chapters – with H. D. Maskus on *Peas and other legume proteins* and independently on *Canola and other oilseed proteins*. The former describes the production and potential of pulses for human consumption and the processing of legumes to produce isolates and their potential uses. Canola protein is a lesser known

oilseed protein and possible uses are reviewed, together with other oilseed products including those from flax and hemp. In terms of the protein recovered from canola, the nutritional quality of this isolate is equivalent to soybean for 10–12 year olds and adults. So this chapter is a timely reminder of their great potential. For example, hydrolysis of these proteins has yielded peptides which have potential health benefits.

Gluten is a proteinaceous by-product produced during the extraction of starch from wheat. It is a relative newcomer as a food ingredient, but now finds more and more applications in the food and non-food sectors, mainly a result of its viscoelastic, thermosetting and water-holding properties. It is the major plant-based protein after soya-based proteins. Li Day gives a very interesting world-wide perspective on this source of protein, its manufacture and utilisation. It is an efficient utilisable source of α-amino nitrogen,
which could meet the demand for the synthesis of non-essential amino acids in the human body. The protein structure is complex but is lucidly explained in this chapter. Physical and chemical modifications are described also. Indeed the author has managed to provide a clear picture of the material and applications of what has been a rather low-key product scientifically. The regulatory section and its relation to coeliac disease is particularly valuable.

"Coagulated potato proteins and hydrolysates thereof as novel food ingredients" received approval by the EC in 2002 and obtained GRAS approval in the USA the same year. Few of us would have dreamt that a protein obtained from potato juice could make such a potential impact on the health food area. A. C. Alting, L. Pouvreau, M. L. F. Giuseppin and N. H. van Nieuwenhuijzen show how this by-product of the potato starch and the French fries industry could move into large-scale production. The main proteins are patatin and serine and cystein protein inhibitors. Much work needs to be done on the emulsification and foam-forming properties but the account is both hopeful and challenging. For both GRAS and EC Novel Food the limits have been set for high dosage food applications for residual sulphite (<100 ppm) and glycoalkaloids (<150 mg/kg product). This is a product we all need to learn more about.

T. J. A. Finnigan describes the production of mycoprotein by fermentation and the conversion of the harvested mycoprotein into meat-like textures. These meat-free products are sold under the Quorn™ brand. Regulatory approval in the UK for unrestricted sale was granted in 1985 after extensive safety studies and thus precedes the EC Novel Foods legislation. In these global warming days it is interesting that the author suggests that Quorn™ mince may have a significantly lower CO_2 emission rate than the production of beef. Such developments might well be considered now as justified in the interests of ensuring future food security.

Ioannis S. Chronakis and Maja Madsen describe the nature and properties of the algal proteins. Their functional and nutritional properties compare favourably with terrestrial plants. They have applications for gelation, water

and fat absorption, emulsification and foam stability. Red and brown seaweeds having relatively low protein contents are utilised as a source of polysaccharides such as carrageenans, agar and alginate. It is interesting to note, therefore, that blue and blue-green seaweeds can consist of as much as 40 to 60% protein. The cleavage or limitation of linkages between polysaccharides and proteins appear to be a determining factor for improving the extraction. It is clear from the authoritative treatment of this subject that there is likelihood of increasing use of these in food. Some is now used in animal feeds. The genetic modification of these materials could lead to important new protein sources. The use of Spirulina-based products for food colouring is already gaining ground in Japan. This is a truly fascinating and emerging area.

"Texturized vegetable proteins", the title of the chapter by Mian Riaz did not convey immediately the extent of this important food protein source. Just as well then to quote the United States Department of Agriculture's definition of textured vegetable protein products for use in the school lunch programme as "*food products made from edible protein sources and characterized by having a structural integrity and identifiable structure such that each unit will withstand hydration and cooking, and other procedures used in preparing the food for consumption*". Essentially these are texturised soy proteins produced by commercial companies in the USA which take up the place of meat in the human diet. The main sources of the protein are oilseed proteins, cereal protein, legume and pulse protein and leaf proteins. The technology and sourcing of these materials is well described in this chapter.

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Caseins

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Abstract: This chapter discusses the commercial methods used to separate and concentrate the casein from the other components in bovine milk. The family of casein ingredients produced are discussed in terms of casein structural and physico-chemical properties which resulted in both the production of the ingredient and functionality of the ingredient in various food products. Casein-based dehydrated food ingredients are discussed in a number of food systems which utilise some of the many functional attributes associated with this natural protein polymer. A section is also included on some of the interactions which occur between the casein ingredient and other components in the food system in question. Finally an approach to technical specifications and an insight into the complex area of regulatory status are included.

Key words: casein, colloids, structure/function, food use, interactions.

2.1 Introduction

Casein is the dominant protein group in bovine milk and is the major functional contributor to a family of dairy ingredients which are used ubiquitously in the food industry. The caseins are nature-designed to be dispersed in an aqueous solvent, carry relatively large quantities of calcium and calcium phosphate and still maintain a low viscosity at ~2.5% (w/w) concentration. The general composition of bovine milk is outlined in Table 2.1. Due to the liquid nature of bovine milk, the dairy industry has the capability of fractionating the components resulting in an array of functional ingredients for application in the food industry. This chapter will concentrate on the methods of separation of the casein components from bovine milk and the many functional attributes that can be generated by inclusion of the casein-based ingredients in food systems.

2.2 Manufacture of casein-based ingredients

The caseins are a heterogeneous group of phosphoproteins (they contain the amino acid phosphoserine) which self-assemble into protein packets we

Table 2.1 Typical levels of the major colloidal and soluble components in bovine whole milk

Component	Colloidal	Soluble
Fat (%)	4.0–4.4	
Casein (%)	2.5–2.8	
Whey protein (%)		0.5–0.6
Lactose (%)		4.4–4.6
Calcium (mg/100 ml)	80	40
Magnesium (mg/100 ml)	3	8
Potassium (mg/100 ml)		150
Sodium (mg/100 ml)		50
Chloride (mg/100 ml)		100
Phosphorous (mg/100 ml)	45	45
Citrate (mg/100 ml)	15	170

recognise as being casein micelles. These micelles range in diameter from 50 to 300 nm and give skimmed milk its white appearance. Colloidal calcium phosphate and bound calcium are intimately associated with the self-assembly process and it is this close association between the mineral and protein components which gives native casein its unique properties. The casein group of proteins are composed of α_{S1}, α_{S2}, β and κ-casein in an approximate ratio of 40:10:40:10. The stability of the casein micelle to heat, ethanol, calcium, etc., is generally ascribed to the presence of κ-casein on the surface, which provides a repulsive force between the individual micelles. Separation of the casein component from skimmed milk is generally achieved using one or other of two processes, namely, precipitation or membrane processing.

2.2.1 Skimmed milk powder (SMP)

This is the major milk-derived powdered ingredient and requires no major separation processes except removing the fat component by centrifugation. Dried skimmed milk produces a powder with ~35% (w/w) protein, with casein contributing ~80% of the total protein. The skimmed milk is evaporated to 45–50% solids prior to spray drying. Skimmed milk powder (SMP) is produced with a number of heat classifications, namely low, medium and high. The degree of heating of the skimmed milk prior to evaporation and drying determines the functionality of the reconstituted powder. This heat-induced change in functionality is, in general, related to the degree of whey protein denaturation and whether the denatured whey proteins are permanently attached to the casein micelle. Reconstitution of SMP for further processing (recombined concentrated milks), such as retorting or UHT, requires that the reconstituted SMP be heat stable and this requires a powder with a high heat classification.

2.2.2 Acid casein

As caseins are polyelectrolytes (polymers with charged side groups), they are very susceptible to changes in pH. They have, in general, an isoelectric point (no nett charge) in the region of pH 4.6. Changing the pH of skimmed milk from ~6.7 (natural pH) to 4.6 causes the caseins to self-aggregate on a macroscopic scale. This pH-related behaviour of the casein component in milk allows for a concentration of the casein and elimination of the other components in the milk (lactose, whey proteins and soluble minerals). The destabilisation of the casein moiety in milk through isoelectric precipitation is the preliminary step to the production of acid casein. Hydrochloric acid is often used to change the pH of milk to the isoelectric point resulting in the formation of a coarse precipitate. However, sulphuric acid has been used and natural fermentation of the milk with *Lactobacillus* sp. to produce lactic acid is often the method of choice. Since all the other components of skimmed milk are soluble at this pH (lactose, whey proteins and minerals, including the colloidal calcium phosphate), it is an efficient method of producing high casein powders. The whey is separated from the curd using decanter centrifuges and this is generally termed acid whey. Subsequent washing with water further purifies the casein component which is pasteurised and dried.

2.2.3 Rennet casein

The action of chymosin (rennet) through hydrolysis of κ-casein to produce para-κ-casein and the glycomacropeptide is the preliminary step in the production of cheese, resulting in the formation of curds and whey. This is the basis to rennet casein manufacture where subsequent treatment of the curd is similar to acid casein manufacture (see Section 2.2.2). The major compositional differences between the two casein ingredients is the absence of intact κ-casein and the presence of colloidal calcium phosphate and bound calcium in the rennet casein ingredient. While precipitation (either using acid or rennet) is an efficient method of producing high casein ingre- dients, the dried protein is by its nature insoluble when reconstituted in water.

2.2.4 Caseinate

Since acid casein functionality is limited due to its low rehydration rate and inherent insolubility, the potential functionality has to be released by pH re-neutralisation through alkali addition. The alkali of choice would include sodium hydroxide, calcium hydroxide, ammonium hydroxide or sodium carbonate. The functionality of a protein ingredient is generally related to its ability to bind water, rehydrate, redisperse or dissolve. The functionality of casein (a polyelectrolyte) is inextricably linked to mineral/ casein interactions which are pH dependent. If the pH of acid casein is

adjusted with sodium hydroxide, the casein becomes negatively charged (the carboxyl groups (COOH) on the amino acids such as glutamic and aspartic acids become deprotonated (COO^-) and are negatively charged) and sodium becomes the counterion (sodium caseinate). This change in pH (increased casein negative charge) disaggregates the insoluble acid casein to a "soluble" translucent dispersion. If, however, the acid casein is pH adjusted with calcium hydroxide, the resulting caseinate (calcium caseinate) is a white colloidal dispersion. While the pH increase encourages the insoluble acid casein to disaggregate, the divalent calcium cation binds to a percentage of the newly created negatively charged side-chains. Due to the divalent nature (2^+) of calcium, it is capable of crosslinking the casein polymers resulting in the formation of small casein aggregates which are stable to sedimentation.

2.2.5 Milk protein concentrates (MPC)

The production of dairy ingredients with enhanced casein levels requires the casein to be concentrated *vis-a-vis* the other components in the milk. The precipitation method has been outlined above but casein can also be concentrated in its liquid state by membrane technology. Milk protein concentrate (MPC) is usually produced using membrane technology and, as its name suggests, contains both casein and whey proteins in the ratio that exists in milk. MPC is available in a range of protein levels from 42 to 85%, the lactose level falling as the protein level increases. The casein fraction is in the native micellar form and therefore carries significant quantities of calcium and phosphate. While the casein component in the micelle is aggregated through calcium or calcium phosphate mediated crosslinks, the crosslinked casein aggregates also transport the insoluble calcium phosphate in nanoclusters suitable for nutritional requirements. MPC can be used for its nutritional and functional properties. The high protein, low lactose ratio makes MPC suitable for protein-fortified beverages and low-carbohydrate foods. The whey protein is usually still in the native form but can be easily denatured prior to processing if required. Milk protein isolate is defined as having at least 90% protein and is the top of the MPC range of ingredients. MPC can also be produced by precipitating the proteins out of milk or by dry-blending the milk proteins with other milk components. The selection of MPC ingredients available ranging from skim milk powder (35% protein), MPC 42, MPC 70, MPC 75, MPC 80, MPC 85 and finally MPC 90, give a selection of compositional and functional attributes as a result of the changing mineral:protein:lactose ratio.

2.2.6 Phosphocasein

This is essentially production of micellar casein as it occurs in milk but with no whey proteins and is generally produced using membrane technology.

Membrane technology utilises retention and permeation principles, the membrane pore size determining whether any particular entity in the milk remains in the retentate or is eliminated in the permeate. Microfiltration results in the elimination of the native whey protein, lactose and soluble salt fractions, retaining the micellar casein with its associated colloidal calcium phosphate. Following microfiltration, diafiltration, evaporation and drying, a 75–80% protein powder is produced. Rehydration of phosphocasein powders is difficult due to the ultra-low level of solutes dissolved in the water phase prior to drying. This results in dried individual casein micelles in an extremely shrunken state, making rehydration difficult. While cold water dispersibility is difficult on rehydration of these powders, heating to >50°C while agitating generally rehydrates the powder adequately. This is also true for high protein MPC powders. Due to the inherent low ionic strength conditions prevailing in phosphocasein dispersions, stability to flocculation at processing temperatures (60–80°C) at pH < 6.7, is limited (Le Ray *et al*., 1998). However, re-introduction of the soluble milk salts in the form of milk ultrafiltrate can restore the inate heat stability of the micellar casein. Phosphocasein exhibits physico-chemical and micellar behaviour similar to milk in terms of particle size, rennet gelation (Pires *et al*., 1999) and acid gelation (Famelart *et al*., 1996).

2.3 Structure and properties

The composition of the various casein-based ingredients is outlined in Table 2.2.

In food formulation, the choice of a casein-based ingredient will depend on the requirement of the food to be formulated. The food will generally need to be heated either to pasteurisation temperatures or higher if retorting or UHT is required. The casein ingredient will need to be stable to

Table 2.2 Composition of casein and caseinates

Component	Acid casein	Rennet casein	Sodium caseinate	Calcium caseinate	Micellar casein
Protein (%)	87–89	81–82	90–92	90–92	80–82
Moisture (%)	9.5–10	10–11.5	3–4	3–4	3–4
Ash (%)	1.6–2.0	7.5–8.2	3.6–4.0	3.6–4.0	7.5–8.0
Lactose (%)	0.1–0.2	0.1	0.1	0.1	0.5
Fat (%)	0.9–1.1	2.0	0.7–0.9	0.7–0.9	1.0
Calcium (%)	0.12–0.3	2.7–3.0	0.15–0.4	1.3–1.5	2.5–3.0
Phosphorous (%)	0.75–0.8	1.5	0.8	0.8	1.5
Sodium (%)	0.07–0.12	0.14–0.26	1.3–1.5	0.1–0.2	0.2–0.3
Potassium (%)	0.18–0.27	0.42–0.6	0.18–0.3	0.18–0.3	0.4–0.6
Magnesium (%)	0.02–0.04	0.02–0.04	0.02–0.04	0.02–0.04	0.02–0.04
pH	4.4–5.2	7.1–7.4	6.6–6.8	6.6–6.8	6.9–7.1

aggregation, viscosity development, precipitation or sedimentation. As outlined in Section 2.2, destabilisation of the casein micelle through manipulation of the pH or enzymatically by rennet addition can be an integral part in the production of high casein-based ingredients. These same principles can be employed in the choice of the casein ingredient in food formulations. The composition of the casein ingredient will determine the initial structure of the casein entity and therefore its functionality. It must be borne in mind that aggregation of casein may be a requirement for some formulations. Therefore aggregation or potential structure formation can be viewed as a negative or a positive attribute depending on whether a liquid or solid end result is required. The ability of complex colloidal mixtures of caseins, fat and minerals to be stable to high heating regimes is an important property of casein-based ingredients. Dehydration of casein-based concentrates is a complex phenomenon whereby water is removed either by centrifugation (acid casein) or evaporation (SMP), prior to secondary removal of most of the residual water using different types of driers (spray driers, ring driers, attrition driers, etc.). Casein micelles can have a voluminosity of ~3.5–3.7 ml of solvent/g casein at their normal pH and concentration at ambient temperatures (pH 6.7, 2.5% w/w and 20°C). This suggests that each casein micelle is approximately 20% casein and 80% solvent. Casein micelles are, in essence, nanogels and as such, have a surface which interfaces with the aqueous co-solvent and a porous interior matrix which may or may not be in equilibrium with the exterior aqueous co-solvent. In milk the co-solvent would constitute a lactose/salt/water solution.

Depending on the production protocol for different casein ingredients, the composition of the aqueous-based solvent changes. For high casein ingredients most of the dissolved solids are removed by washing with pure water thus changing the quality of solvent *vis-à-vis* the casein. It must also be realised that the voluminosity of casein particles can change depending on the co-solvent, co-solvent pH and temperature. Temperature and pH are major influential factors determining the internal volume of the casein micelle and may markedly affect the structure of food systems containing high concentrations of casein (>20% w/w). In the case of temperature, especially, and within certain limits of concentration (<30% w/w), this can be viewed as a reversible phenomenon. As intimated in Section 2.2, the structure of the various casein ingredients depends on mineral/casein interactions. In general, the ratio of the various caseins remains constant (except rennt casein where κ-casein has been hydrolysed) across the various casein ingredients, the mineral level and type will determine the structure. Sodium caseinate could be viewed as the smallest casein entity we see in aqueous solvents. It has dimensions of ~10 nm and can be visually clear when any residual fat is removed. It is viewed as a very efficient emulsifier of oils and fats due to its balance of hydrophobic and hydrophilic amino acids and rela- tively small size. Calcium caseinate, however, has a particulate structure which affects its functional behaviour.

Phosphocasein and calcium caseinate are small colloidal particles and the net interactions can be either repulsive or attractive. When the interactions are repulsive, the colloid is stabilised while attractive interactions can lead to flocculation, aggregation or gelation. Depending on the pH, the negative charge on the casein colloidal surface can result in charge repulsion which helps to stabilise the colloidal dispersion. However, as we reduce the pH, the negative charge is reduced and the system becomes less stable. A second important repulsive interaction is steric stabilisation, which occurs when colloidal particles have a short polymer attached by one end to their surface. In casein micelles and calcium caseinate this steric stabilisation is conferred by κ -casein, which is removed on renneting.

Due to their porous particulate structure (phosphocasein, SMP, calcium caseinate) and their capacity to control the solvent internally, there is a critical concentration of protein that can be dispersed in an aqueous solvent before the dispersion goes from liquid to viscoelastic. This is the concentration region where the colloidal particles are crowded or jammed and lose their ability to move unhindered. This becomes obvious during the production of SMP where water removal using evaporators stops at ~55% solids. At solids concentration greater than this level, the propensity for solid-like behaviour is great. This change from liquid to solid-like behaviour is an important area of interest for both processing of casein-based ingredients and their application into food systems. The ability to change the critical concentration where the transition occurs through control of the solvent quality through temperature, ionic strength and pH intervention would progress our understanding of both the dehydration process in the production of the ingredient and the rehydration of the ingredient for application in various food systems.

Foods can include emulsions with a variable fat content. They can be water continuous such as yoghurt or oil-continuous such as table spreads. The strongly amphipathic nature of proteins, particularly casein, resulting from their mixture of polar and non-polar side chains, causes them to be concentrated at interfaces. At fluid/fluid interfaces, caseins may change their tertiary structure, existing in extended configurations with hydrophobic side chains orientated towards the non-aqueous phase and hydrophilic side chains directed towards the aqueous phase. The source and conformation of the dairy-based protein emulsifier used to stabilise emulsions in foods can have great effects on texture. As the casein-based emulsifier changes from sodium caseinate to calcium caseinate to calcium phosphate caseinate, the properties of the resulting emulsions markedly change, due to the differing functionalities of the emulsifiers themselves. While not mentioned in Section 2.2, calcium phosphate casein is meant to denote a reassembled casein micelle-like particle produced from acid casein by manipulation of the pH through judicious use of calcium chloride, sodium phosphate and sodium hydroxide.

2.4 Uses and applications of casein-based ingredients

Different food systems are composed of varying ratios of proteins, fat, sugars, salts and water. Both the ratios and the concentration of these components determine the structure and texture we recognise as foods. They may be liquid, semi-solid or solid, depending on the concentration and type of ingredient. They may also contain permitted additives such as emulsifiers, stabilisers, colours, flavours, etc. The structure, texture and stability of any food which contains casein will be affected by pH, ionic strength and temperature. This will also be applicable to emulsions stabilised by casein.

2.4.1 Acid gels

Casein-based ingredients are often added to milk to control the behaviour of the acid gel formed on fermentation using bacterial cultures. These would include yoghurt-type, cream cheese, etc. Aqueous dispersions of sodium caseinate, calcium caseinate, micellar casein and SMP, at standard casein concentration, will all form acid gels when acidified slowly with glucono-delta-lactone. This is not surprising given the production profile of acid casein (Section 2.2). However, their gelation kinetics are different. The pH where gelation is initiated (gel point) is determined by temperature and ionic strength of the solvent. Fermentation of casein containing dispersions can be viewed as a pH/heat stability profile where the temperature is 40°C. The gel point thus is the interaction between temperature and pH. When unheated SMP dispersions are acidified, they form a weak gel at pH ~5; however, when micellar casein is acidified it gels at pH 6–6.2. This phenomenon can be generalised as casein micelles are unstable to heat and pH reduction when the ionic strength of the aqueous phase is too low (Auty *et al*., 2005). The SMP has its natural level of soluble salts, hence acid gelation is inhibited, while micellar casein, calcium caseinate and sodium caseinate dispersions have very low inate salt levels. This suggested that textures of foods from dairy sources or containing casein ingredients are to a large extent dependent on the soluble salt content.

2.4.2 Recombined milk products

While SMP contains only 35% protein, 80% of the protein is casein and its properties are controlled to a large extent by the casein component. SMP or its fat-filled counterpart are widely used for recombined milk products where stability to secondary heating is paramount. These recombined products are usually retorted in cans or UHT treated to ensure long life. A complex sequence of events must occur prior to dehydration of the milk to produce a SMP which on reconstitution to high solids (20–31%) will be stable to secondary heating at elevated temperatures (120 or 140°C). At

low concentrations of milk solids (10% solids) most milk samples are stable to high heating regimes, provided the pH is in the normal range (pH 6.55–6.8). However, when milk is concentrated (>20% solids) it has reduced heat stability across the pH range 6.2–7.2. Besides the casein micelle and its internal complement of calcium and calcium phosphate, the whey proteins also play a critical part in both stabilising and destabilising the reconstituted SMP on subsequent heating. Since some or all of these components are markedly unstable to high heating regimes in isolation, the ability of the components to self-protect one another is central to heat stability. To protect the reconstituted SMP from coagulation on secondary heating, the milk, prior to dehydration, must be preheated to high temperatures (120°C for 2 min), evaporated and spray dried. This heating step not only ensures denaturation of the heat-labile whey proteins but also their degree of aggregation. The pH of the raw milk prior to preheating will determine the degree of whey protein interaction with the colloidal casein particle. Heating at pH acidic to the natural pH results in more whey protein attached to the casein, while heating at pH alkaline to the natural pH results in more denatured whey protein in the serum (not attached to casein). While phosphates and/or citrates are used to aid in heat stability, the preheating step is dominant. Of crucial importance to understanding this complex phenomenon, which we casually call heat stability, is the heat coagulation time/pH profile. This can also be viewed as a viscosity/pH profile depending on the methodology used to assess it. Either way, where maximum stability is established the pH should be the natural pH of the reconstituted powder.

2.4.3 Food emulsions

Food emulsions are generally of the oil-in-water or water-in-oil type but occasionally multiple emulsions are utilised. The oil-in-water emulsion would be typified by cream liqueur, ice-cream or mayonnaise, while the water-in-oil type, although less prevalent, would be represented by table spreads. A suitable emulsifier is a prerequisite for efficient emulsion formation and these can include proteins and low molecular weight surfactants. The most widely used protein emulsifier for oil-in-water emulsions in the food industry is probably sodium caseinate. However, other casein-based emulsifiers (calcium caseinate, skimmed milk powder) are also used for this purpose. The production of oil-in-water food emulsions utilises a lot of energy in creating an oil surface which is stabilised by the adsorbed protein. This is generally performed using high pressure homogenisers (valve-type) although crude emulsions can be produced using mixers of various types and nano-emulsions can be produced using microfluidisers.

Long-term stability of emulsions depends in part on the thickness and strength of the adsorbed films at the oil-water interface (Dickinson and Stainsby, 1982). Dairy-based primary emulsifiers (caseins) are available in

a number of different states which can have a dramatic effect on both the efficiency of emulsification and the subsequent functionality of the emulsion. These can range from the apparently simple sodium caseinate, which is the most disaggregated of the casein-type emulsifiers, to the more aggregated calcium and calcium phosphate caseinates, which can increase the protein load on the oil-water interface. It must be borne in mind that the properties of emulsions are largely dependent on the properties of the interfacial layer (the fat type will play a part) which are dependent on the environmental conditions pertaining in the aqueous phase. Ice-cream is a frozen aerated oil-in-water emulsion where micellar casein and whey proteins can act as emulsifiers, while coffee-whiteners are oil-in-water emulsions where caseinate may be the preferred emulsifier. Acidic milk-based emulsions may rely on the emulsified oil droplets being an integrated part of the gel structure through interaction of the interfacial layer with the bulk casein/whey protein complexes.

The principal ingredients of table spreads are fat (dairy or vegetable), fat-based emulsifier, milk protein, stabiliser, sodium chloride and water, and each of these will affect the emulsion, processing and consumer behaviour of the final product. The level of sodium chloride in the aqueous phase can vary but is usually in the region of 1.5%, w/w. The water-in-oil pre-emulsions of fat spreads are always stabilised by high shear working of the emulsion at low temperatures to a plastic consistency. Before this solidification step, emulsions can become unstable due to either phase separation or phase inversion (Mulder and Walstra, 1974). It is evident that the likelihood of phase inversion increases as the fraction of added disperse phase is increased. It has been suggested that the higher the aqueous phase viscosity, the greater is the stability to inversion (Platt, 1988). Sodium caseinate is often the protein of choice to aid in the stabilisation of the water-in-oil emulsion. However, buttermilk powder and SMP are also used. While the introduction of NaCl into the aqueous phase was initially for organoleptic reasons, the interaction between NaCl and caseinate also has a significant effect on the stability of the emulsion prior to solidification. The viscosity of a caseinate solution is an indicator of the degree of bound water absorbed by the hydrophilic groups as well as the water trapped inside the aggregated molecules (Korolczuk, 1982). Sodium caseinate contributes to the stability of the water-in-oil emulsion through steric and water binding effects (Keogh, 1992). The same author concluded that NaCl made a significant contribution to the aqueous phase viscosity. While final emulsion stability and its stability to inversion (e.g. becoming an oil-in-water emulsion) may be related to the viscosity of the caseinate-based aqueous phase, the interaction between the level of fat soluble emulsifier and the aqueous caseinate may also be significant (Barfod *et al.*, 1989). Significant reductions in the level of NaCl in the aqueous phase can lead to inversion problems during processing, and alternative methods of increasing the aqueous phase viscosity may have to be approached.

2.4.4 Cheese analogue

Rennet casein is a major ingredient in cheese analogue production but to exhibit the required functionality it has to rehydrate in water to relatively high concentrations. As indicated in Section 2.2, rennet casein is produced by hydrolysis of κ -casein yielding an insoluble curd. To make this dried curd dispersible, the components responsible for insolubility must be either removed or modified. Calcium chelating salts, or "emulsifying salts" as they are termed in the processed cheese industry, are used to disaggregate the casein polymers through chelation of casein bound calcium and/or calcium phosphate. This removal or modification of the cement holding the casein polymers in a shrunken state results in casein hydration and a return to viscous-type behaviour.

2.4.5 Cream liqueur

The ability of milk to withstand the addition of significant quantities of ethanol without destabilisation has often been used as an indicator of milk quality. The relationship between milk constituents and ethanol stability was studied by Davies and White (1958) and the importance of pH and the composition of the milk aqueous phase was shown by Horne and Parker (1980, 1981a, 1981b). The main effect of increasing the ethanol concentration in aqueous ethanolic mixtures is a major shift in the dielectric constant which decreases the solvent quality (makes it more hydrophobic), thus affecting the solubility of ionic species.

A study by O'Connell *et al*. (2001) emphasised the importance of temperature on the structure and behaviour of casein micelles in ethanolic solutions. They showed that the repulsive forces between caseins increase and solvent quality is enhanced with increasing temperature, which results in swelling of the micelle and eventual dissociation. They ascribed this behaviour to a dramatic decrease in cohesive interactions between casein molecules on heating in the presence of alcohols to be a consequence of a reduction in phosphoseryl-mediated cross-linking and an increase in protein hydrophilicity. These parameters impinge in the study of cream liqueur systems and the remarkable ability of sodium caseinate to stabilise the emulsion in ethanolic solutions.

Large quantities of dairy ingredients are utilised in the production of cream liqueurs. These would include the cream itself and the primary emulsifier of choice, sodium caseinate. As mentioned above, sodium caseinate is the sodium salt of acid casein and is a very efficient emulsifier. A typical composition of a cream liqueur is shown in Table 2.3. Essentially it is a dairy fat-based emulsion dispersed in an aqueous ethanol sucrose solvent. The fat content can vary from 5 to 16% (w/w) and these emulsions must be stable to separation problems for long periods of time. Most of the problems observed in commercial cream liqueurs revolve around the fat fraction. Creaming, cream plug formation, flecking and gelation are all related to fat

Table 2.3 Typical levels of the major ingredients in a cream liqueur formulation (12% fat)

Ingredient	%
Cream (40% fat)	30
Sugar	20
Sodium caseinate	2.5
Ethanol	14
Trisodium citrate	1.0–1.5
Colours and flavours	~0.1
Water	31.9–32.4

globule destabilisation. However, the fat globules are emulsified by a surface layer of caseinate (Banks *et al.*, 1981a) which confers the sought-after stability in the first place, so destabilisation may be a caseinate problem. Calcium-induced aggregation was considered to be the dominant factor controlling the shelf life of cream liqueurs at high ambient temperatures (Banks *et al.*, 1981b), but other protein interactions might also be important when calcium chelators are present. The addition of trisodium citrate at levels normally present in milk (10 mM) reduced the viscosity at high ambient temperature by reducing the ionic calcium level coming from the cream (Banks *et al.*, 1981b). While sodium caseinate is a relatively expensive ingredient, attempts to substitute it for a cheaper alternative have not had much success. This is mainly due to its high emulsification potential and stabilising power in aqueous alcoholic solutions. Calcium caseinate or phosphocasein cannot readily be used to stabilise the emulsion in cream liqueurs due to their aggregating tendencies in aqueous ethanol mixtures. The resulting cream liqueur would be highly aggregated with the associated increase in viscosity, while it remains liquid where sodium caseinate is the emulsifier choice. Again, this indicates the importance of the structural arrangement of the individual casein polymers, which are mineral crosslinked, on the subsequent behaviour when the solvent quality is altered. However, sodium caseinates from different sources have been shown to have different alcohol stabilities which were attributed to different production protocols and different levels of damage to the protein during processing (Muir and Dalgleish, 1987).

O'Kennedy *et al.* (2001) showed that pH, ionic strength and ethanol content play a significant part in determining the stability of sodium caseinate to aggregation and eventual precipitation. They also concluded that about one-third of the casein protein was susceptible to ethanol induced aggregation, the balance remaining in a non-aggregated state under the conditions of pH, ionic strength and ethanol concentration used (pH 7, 25°C). The main proteins susceptible to ethanol induced aggregation were the sulphydryl containing α_{S2} and κ-casein fractions. The non-aggregated

casein fraction showed no time-dependent increase in viscosity of cream liqueur analogues at 45°C over a 63-day period. An opportunity, therefore, exists for the development of a caseinate ingredient for the cream liqueur industry based on that fraction which does not aggregate in the presence of ethanol. Lynch and Mulvihill (1997) have shown that the apparent viscosity of cream liqueurs on storage at 45°C was dependent on the sodium caseinate source and the authors suggested that electrostatic and sulphydryl interactions were involved.

2.4.6 Chocolate

Milk chocolate is an integral part of the confectionary area and utilises large quantities of dried dairy-based ingredients. These are mainly composed of WMP and chocolate crumb but SMP and whey powders are also used.

In this particular application of casein containing products a mental shift is required as we are used to dealing with milk protein functionality or sugar behaviour in an aqueous environment. Chocolate is a fat continuous system (contains very little water (0.1%)) composed of cocoa butter (mainly) with a dispersed phase of solid particles. What is the relevance of casein in a food product that contains no water? In this case the casein micelles have already been dehydrated prior to incorporation into the fat phase or they have been dehydrated in the presence of cocoa butter and cocoa liquor in the production of chocolate crumb. Generally these powders are roller refined to a particular size when dispersed in the fat phase. A typical composition of milk chocolate is outlined in Table 2.4. The refiners reduce the particle size of the sugar and casein-based powder yielding a flake-like confection. While the gross composition of this refined flake is not unlike the finished chocolate, the refined flake has no propensity to flow when heated above the melting point of the cocoa butter. Following the refining step for particle size reduction, the flaked mixture is conched to remove a certain amount of residual water and a significant amount of volatiles which affect the flavour. The conched mass becomes liquid when lecithin is added at temperatures above the melting point of the fat. This suggests that the dispersed SMP/WMP and/or the sugar particles interact with one another in the fat phase. Whether the SMP is responsible entirely for this phenomenon is

Table 2.4 Typical levels of ingredients in milk chocolate

Ingredient	%
Cocoa butter	20
Whole milk powder	24.4
Sugar	43
Cocoa liquor	12.4
Lecithin	0.2

uncertain. This phenomenon is central to the rheology of milk chocolate, allowing the insoluble solid particles to flow over one another when dispersed in triglyceride oil. However, when the milk chocolate enters the mouth and the cocoa butter melts at body temperature, the casein micelles, albeit in an altered state after considerable abuse (Maillard, caramelisation), rehydrate in aqueous phase of the saliva.

2.4.7 Bakery

SMP and whole milk powder are often used in bakery products but rarely are the high casein-based powders utilised. Current research aims to completely substitute gluten with a functional high casein-based ingredient (Stathopoulos and O'Kennedy, 2008). The principle behind this approach is that by increasing the calcium concentration to an optimum level in the casein/caseinate ingredient it will be possible, under the correct pH and ionic strength conditions, to replace the highly functional (covalent) S-S bonds in a gluten-based dough with calcium-induced casein-casein complexes.

2.5 Interactions with other ingredients

The use of polysaccharide-based biopolymers in formulated foods is a common occurrence. The reasons for using such ingredients might include viscosity control, water control or general stabilisation of an emulsion from creaming. These might include guar gum, locust bean gum, carrageenan, alginate or starch. When colloidal casein and emulsions stabilised by colloidal casein come in contact with polysaccharide-based biopolymers, a number of things could happen depending on the polysaccharide chosen. If the polysaccharide is a neutral polymer (no electrical charge on the backbone) such as guar or locust bean gum, then phase separation will occur. On aging this mixture separates into two distinct liquid phases, one containing the emulsified fat and the colloidal casein and the other containing mainly the polysaccharide. This can be obvious in some ice-cream formulations where gross separation of the phases can occur on aging overnight. This is not necessarily a bad thing as the phases remix readily on stirring and the mixture is aerated and frozen which prohibits any gross separation. However, it is important to be aware that the properties of the mixture can be determined by the volume of water associated with each phase. If the polysaccharide phase is dominant (>50% of the volume), then the properties of the mixture will be dominated by the polysaccharide. This can be viewed as a water-in-water emulsion with one water phase dispersed in the other depending on which phase is dominant. In ice-cream the ratios of the phase volumes become very important as both phases will freeze separately and ice crystal size may be affected.

If the polysaccharide is positively charged, such as chitosan, then gross precipitation of the colloidal casein will occur. Even when both polysaccharide and colloidal casein are negatively charged (κ -carrageenan) precipitation can occur. It is thought that the negatively charged carrageenan interacts on positively charged patches on the colloidal casein.

The addition of large concentrations of soluble sugars may also directly affect the behaviour of the colloidal casein ingredient. Significant concentrations of sucrose have been shown to affect basic colloidal casein properties such as acid gelation and rennet gelation. Schorsch *et al*. (2002) have shown that sucrose addition promoted acid gelation but inhibited rennet gelation of micellar casein in a milk salt solution. This behaviour was interpreted as a change in solvent quality affecting the surface behaviour of κ -casein which in turn resulted in a different expression of casein behaviour.

However, one of themes running through this chapter, namely the internal volume of porous colloidal particles, is of extreme importance in the interpretation of rheological behaviour of casein-containing food systems. When the concentration of a solute in the aqueous solvent increases to such an extent as to be a highly significant part of the co-solvent, what is the composition of the co-solvent in the internal volume of the colloidal particle? This could be aqueous lactose in the case of evaporated milk, aqueous ethanol as outlined in the previous section or aqueous sucrose as mentioned above.

2.6 Technical data and specifications

Specifications, both compositional and descriptive are an important snapshot of any ingredient and are an indicator of reliability. However, food formulators who buy in casein-based ingredients should be aware that ingredient suppliers may not write specifications for specific products. They may of course have technical help in the application of casein in different food products. However, as outlined in the preceding sections, casein behaviour can change dramatically when dispersed in co-solvents containing added sugars and salts. The main points to note for the various casein-based ingredients including SMP, acid casein, rennet casein, caseinates, MPC and phosphocasein are outlined below in the form of questions. While SMP production is fairly routine, it may vary in composition throughout the season or lactation depending on where it is produced. The heat classification should be specified as different applications require different powders. Should SMP for supplementation of milk for yoghurt be of a certain heat classification if the yoghurt producer is going to heat the supplemented milk anyway? Should acid casein have a specific pH (i.e. pH 4.6) or is the pH spread 4.0–5.0 acceptable? Should rennet casein have 10% moisture or is a higher moisture content (i.e. 15%) more acceptable from a dispersibilty

and conversion point of view? Is sodium caseinate which is spray dried more acceptable than dry blended acid casein and alkali?

2.7 Regulatory status

The regulatory status of dried milk products is a complex area which can change from one jurisdiction to another. These include European Union Legislation, United Kingdom Legislation, United States Legislation and from an International perspective (Codex Alimentarius). A detailed review of the complexity of the regulations was undertaken by Hickey (2009) and should be consulted where necessary.

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3

Whey proteins

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Abstract: Whey protein ingredients have become increasingly important in formulated foods over the past 30 years. Whey proteins are usually supplied as whey protein concentrates (80% protein) and whey protein isolates (90% protein). Whey proteins are a by-product from processing of other dairy products, notably cheese and casein, and attention must be paid to the origin of the whey as this affects both protein composition and mineral composition of the ingredient. Whey proteins are important nutritionally as a balancer for other proteins in specific nutritional applications, such as infant formulae, and on their own as a rich source of branched-chain amino acids, important in muscle nutrition. Whey proteins also have important functional benefits, particularly because of their gelling and water-binding capacity, and also because of their ability to stabilise interfaces in foams and emulsions. A range of new technologies currently being explored may give rise to a range of novel whey protein ingredients with enhanced functionality; however, the long-term sustainability of whey proteins as food ingredients is a matter of concern.

Key words: whey protein concentrate, whey protein isolate, beta lactoglobulin, alpha lactalbumin, functional proteins, protein nutrition, branched-chain amino acids, satiety, gelation, foaming, emulsification.

3.1 Introduction

Commercial food ingredients manufactured from whey proteins first made their appearance in the last two decades of the 20th century, and have become widely used for their nutritional and functional properties. By the end of the first decade of the 21st century, they have evolved to a range of sophisticated, targeted food ingredients with a range of functional and nutritional properties. This chapter will cover the background and use of modern whey protein products from cows' milk products, i.e. from *Bos taurus*. Although whey protein products have been produced from the milk of other species, they are minor niche products.

Early whey protein products were made largely using membrane processing to reduce the level of lactose in the whey before drying and had protein contents of 34%, 56% and 70%, and some of these are still commercially available. As technology improved and standards were developed for regulatory and customs purposes, whey protein concentrates have been standardised at 80% protein, while the more pure whey protein isolates are typically 85% or 90% protein, and these are the products covered in this chapter.

3.2 Manufacture of whey protein ingredients

The protein composition and functionality of whey protein products reflects the composition of whey proteins in the milk, but is modified by the processing that has been undertaken to produce the whey, and then to manufacture the protein concentrate from it. Whey is produced as a by-product of the manufacture of other dairy products, particularly cheese and casein, and has thus been through the processes involved in producing those products from milk. Because all the major whey protein species are globular proteins with defined tertiary and often quaternary structure, they are susceptible to denaturation by heat treatments. Most whey will have undergone several heat treatments of differing severity before manufacture of whey proteins even begins.

3.2.1 Effect of source of whey protein

Whey protein products reflect the whey protein composition of the milk they were produced from. This means that the product can vary as a consequence of on-farm circumstances. In practice, this does not make a lot of difference because whey protein products combine the milk from large numbers of cows on large numbers of farms. The beta lactoglobulin protein has two common genetically determined forms (known as polymorphisms or variants) and it is, in principle, possible to breed herds that contain a single variant. The two variants, known as the A and B variants, have somewhat different properties and are produced at somewhat different levels in the milk due to a variation in the control region of the gene (Lum *et al.*, 1997). The two variants denature differently, affecting functional properties such as gelation (Foegeding *et al.*, 1999). Breeding for the B variant of beta-lactoglobulin was carried out by the Kaikoura Dairy Co-operative in the 1990s (Boland and Hill, 2001); however, the programme was abandoned when the company was amalgamated into Fonterra, and single variant whey protein products have only been made at laboratory scale.

In regions where dairy farming is seasonal, such as in New Zealand, there are small seasonal variations, particularly in the content of immunoglobulins in the early season, and increased serum proteins (particularly serum albumin) in late season.

3.2.2 Effect of manufacturing process

Whey protein products from milk that has undergone a renneting process (sweet whey), i.e. whey proteins from cheese whey or from rennet casein whey, differ from acid whey protein products in that they contain relatively high levels of the caseinomacropeptide, produced when the rennet enzyme cleaves kappa-casein. Whey proteins produced as a by-product of acid caseins (acid whey) contain much lower levels of this peptide (Table 3.1). Whey proteins produced as a by-product of lactic casein will additionally contain components from the lactic acid bacteria used to produce the lactic acid, notably bacterial proteins, polysaccharides and nucleic acids.

Whey protein composition will reflect not only the source of whey, but also the method used to concentrate the protein before drying (Elgar *et al*., 2000). Most modern whey protein products have been purified to 80% or more protein, by one of two methods:

- Membrane processing is the most widely used process to produce 80% whey protein concentrates and some whey protein isolates. This process involves ultrafiltration using a membrane, typically with a molecular weight cut-off of around 10,000, to remove water, lactose and minerals. Because the molecular weight-based separation is not precise, this method can result in loss of some of the smaller proteins, particularly alpha-lactalbumin. For this reason, some membrane-produced whey protein concentrates contain lower levels of alpha-lactalbumin than might otherwise be expected. Higher protein level whey protein isolates can be made by ultrafiltration followed by diafiltration.
- Ion exchange processing is used to produce many whey protein isolates and involves separation by binding the protein to an ion exchange medium and washing out the lactose and minerals, and then washing out the protein using a change of ionic environment and/or pH. This method can produce a very pure protein product, but will modify the protein composition of the product, retaining only proteins with like charge (and acidic isoelectric points), thus largely removing the caseinomacropeptide from sweet whey WPI and biasing the composition to high levels of beta lactoglobulin (80% of total protein in the example shown in Table 3.1).

3.2.3 Effect of heat treatments

Heat treatments during processing of whey proteins can be many, and will always include, as a minimum, pasteurisation of the raw milk and heating during drying. Additional thermal treatments are likely, particularly if the whey has to be stored for any length of time, to control microbial growth. These heat treatments are generally at a level where denaturation of beta-lactoglobulin and alpha-lactalbumin is minimal, but immunoglobulins and serum albumin can be expected to be substantially denatured. A study comparing different heat treatments of milk prior to making whey protein

Table 3.1 Composition of whey protein from different sources

Protein[1]	α-Lac	β-Lg	BSA	IgG	PP	CMP
Whey proteins in unprocessed whey						
Acid whey	0.76 (15)	3.1 (61)	0.15 (3)	0.37 (7)	0.45 (9)	0.25 (5)
Whey protein concentrates (nominally 80% protein)						
Lactic WPC	12 (19)	40 (65)	2.0 (3.3)	3.4 (5.5)	1.9 (3.1)	2.3 (4)
Mineral acid WPC	12 (18)	43 (65)	2.2 (3.3)	5.6 (8.4)	2.5 (3.7)	1.4 (2)
Cheese WPC	11 (18)	**31 (51)**	1.1 (1.8)	3.3 (5.4)	1.5 (2.5)	**13 (22)**
Whey protein isolates (from cheese whey)						
Microfiltered WPI	13 (16)	41 (51)	1.2 (2)	3.5 (4)	5.1 (6)	**17 (21)**
Ion exchange WPI	11 (12)	**71 (80)**	2.6 (3)	3.3 (4)	**0.3 (0.3)**	**0.4 (0.5)**

Values are expressed as % w/w in powder, mg/ml in whey and % of measured protein (in parentheses). Values were determined by reverse-phase HPLC. Data from Elgar *et al.* (2000) rounded to 2 significant figures. Values that are substantially different are highlighted in bold.
[1] Abbreviations: α-Lac alpha lactalbumin: β-Lac beta lactoglobulin; BSA bovine serum albumin; IgG Immunoglobulin G; PP proteose peptone; CMP caseinomacropeptide.

(as demineralised whey powders) found that there were no significant differences between high heat and low heat products for the functionalities measured. These included degree of denaturation, viscosity, water binding capacity, emulsifying capacity and emulsion stability (Outinen *et al.*, 2010). Generally, heat treatments during the processing of whey protein products (as distinct from the processes that may be used to produce the whey in the first place) are considered to have minimal effect on the whey proteins (de la Fuente *et al.*, 2002). Processing to retain the immune capacity of immunoglobulins has been described and requires especially low temperatures (Bounous and Gold, 1991).

3.2.4 Changes during storage of dry powder

Whey protein products are almost invariably supplied as dry powders. During storage, whey protein powders can undergo limited "dry" Maillard reactions to produce lactosyl lysine derivatives of the lysine side chains of beta lactoglobulin (Higgs and Boland, 2009). This derivatisation is slow, occurring over several months depending on the water activity of the powder and the temperature of storage. Generally, storage below 30 °C avoids significant change. The total extent of derivatisation appears to be 3 lactulosyl lysine side chains per beta-lactoglobulin molecule, less than the possible total of 15 (and less than the amount seen with lactosylation in solution). The mechanism of this reaction is not fully understood. This derivatisation is considered undesirable because it renders the lysyl residues non-bioavailable, thus compromising the nutritional value of the whey protein. This is not a problem if whey proteins are a sole protein

source because they are rich in lysine and a 20% loss is inconsequential; however, when whey proteins are used as a balancer for proteins poor in lysine (such as cereal proteins), this loss of lysine can be an issue. The effect of lactosylation on functionality is discussed later in this chapter.

3.3 Chemistry of the major whey proteins

The most important protein in all whey protein concentrates and isolates is beta-lactoglobulin; it makes up about half the whey protein in natural cows' milk, or about 12% of the total protein, and can be increased by processing to form whey protein concentrates and isolates, which may leave behind parts or all of the other proteins (see Table 3.1). An understanding of the chemistry of beta-lactoglobulin will thus go a long way to explaining the chemistry of whey protein products. Key properties of the important whey proteins are given in Table 3.2.

3.3.1 Chemistry of beta-lactoglobulin

Beta-lactoglobulin is a globular protein of the lipocalin family (Fig. 3.1). It has a molecular weight of 18,300 and comprises 162 amino acid residues, including a relatively high proportion of branched-chain amino acids (BCAAs). Beta lactoglobulin contains 22 Leu, 10 Ile and 9 Val (10 in the A variant) residues in the molecule, making it one of the richest known food sources of these amino acids, and giving it some strongly hydrophobic regions. It has a globular structure that includes seven major strands of beta pleated sheet structure that forms a "calyx" or goblet shape, typical of the lipocalins (Fig. 3.1). The cavity formed in the protein can be the binding site for a range of low molecular weight compounds, including fatty acids and retinol (Kontopidis *et al*., 2002) and can bind hydrophobic flavour compounds, leading to flavour defects. It also has a small section of alpha helix that sits against the side of the calyx. The protein normally occurs as a non-covalently linked dimer at neutral pH, but separates into monomers at low pH. A significant feature of beta-lactoglobulin is the sulfur chemistry of this protein: in addition to two internal disulfide bridges that stabilise the protein, there is a single sulfhydryl group at Cys 121 that is buried in the protein, protected by the alpha helix. If exposed as a result of heating or other disruption of the secondary and tertiary structure of the protein, this sulfhydryl can react with other sulfhydryl groups, leading to disulfide exchange and cross-linking reactions with other beta-lactoglobulin molecules or other whey or food proteins, depending on the environment (Creamer *et al*., 2004).

This sulfur chemistry is a key to many of the functional properties of beta-lactoglobulin, as the disulfide interchange prevents renaturation of the

Table 3.2 Properties of whey proteins

Property	Beta lactoglobulin	Alpha lactalbumin*	Serum albumin	Immunoglobulin G	Caseino-macropeptide*
Molecular weight (kDa)	18.2	14	65	180	6.7
No. of subunits	2	1	1	2 light + 2 heavy	n/a
No. of amino acids	162	123	583		64
Isoelectric pH	5.4	4.4	5.5	5.1–8.3	<3.8**

* Both alpha-lactalbumin and caseinomacropeptide are commonly glycosylated, and this is not taken into account in the molecular weight.
** Isoelectric point varies with genetic variant and with patterns of phosphorylation and glycation (Nakano and Ozimek, 2000).

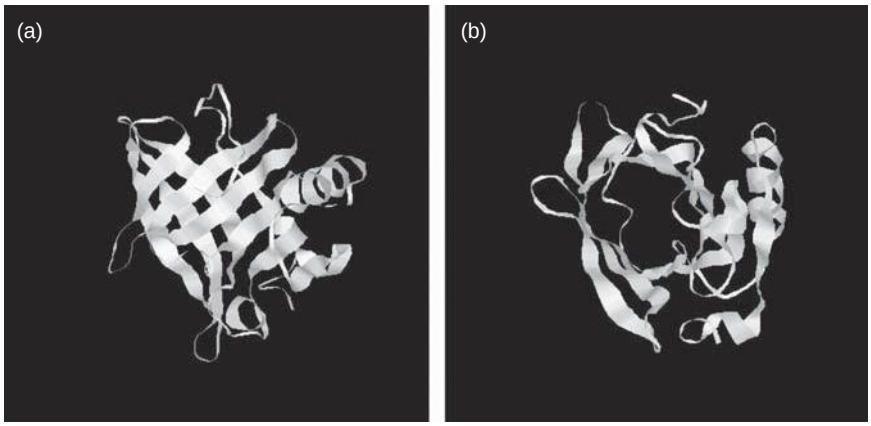


Fig. 3.1 Structure of beta lactoglobulin, (a) showing extensive parallel pleated sheet structure, with alpha helix at right, and (b) looking through the calyx.

unfolded protein, leading to new structures. The high proportion of hydrophobic residues in the protein is also important for its functional properties. When the extensive beta-sheet structure is broken down, for example under heating, the hydrophobic sequences must re-anneal to form new structures that stabilise the hydrophobic groups in aqueous solution. It is generally thought that disulfide cross-linking is the main basis of gel formation, and in investigations where disulfide formation was prevented, gel fracture properties were greatly altered (Errington and Foegeding, 1998). In contrast to this, recent investigations appear to have shown that the properties of whey protein gels are to a significant extent due to hydrophobic interactions. When gels were formed in the presence of disulfide reducing agents or N-ethyl maleiimide, which blocks sulfhydryl groups, there was a relatively minor effect on gel properties; however, the presence of SDS, which breaks down hydrophobic linkages, led to very poor gel properties (Havea *et al*., 2009).

The reasons for the apparent discrepancy between these sets of results are unclear; however, there are important differences between the studies: in the study of Errington and Foegeding (1998) large-scale deformation rheology was used, while the study of Havea *et al*. (2009) used small-scale deformation (oscillating) rheology, so the two studies were measuring somewhat different properties. In the study of Havea *et al*. (2009) the loss of gel strength in the presence of SDS, suggested by the authors to limit gelation due to blocking of hydrophobic bond formation, may alternatively have been due to changes in solubility and/or electrostatic repulsion, rather than due to a lack of effect of disulfide bonding, possibilities that were not excluded by the study.

3.3.2 Chemistry of alpha-lactalbumin

Alpha-lactalbumin is a small protein of MW 14,200 that forms part of the lactose synthase enzyme, and so is necessarily present in all milks. The amount of lactose in milk has been found to be directly correlated with the amount of alpha-lactalbumin, The structure is well stabilised by disulfide bridges, which means that on its own it can unfold when heated and refold to something close to its native form. In the presence of other reactive proteins, such as beta-lactoglobulin, cross-links between the proteins can be formed leading to instability (Gezimati *et al.*, 1997).

3.3.3 Chemistry of immunoglobulins

The immunoglobulins (Igs) in cows' milk are a mixture of blood Igs and Igs manufactured in the mammary gland. The levels of Ig in milk vary as a function of the cow's health and also seasonally. Ig levels are very high in colostrum (as much as 60 g/l), and are probably the main source of health benefits from colostrum. These high levels make colostrum and whey from colostrum particularly difficult to process. Following calving, the level of milk Ig drops over several days. Typical levels in cows' milk are around 0.5 g/l, made up of mostly IgG, with traces of IgA and IgM, usually at around 1/10 the level of IgG. Levels are increased in whey protein products to 4–8% of total protein (Table 3.1).

The Igs have the usual immunoglobulin structure, comprising two heavy chains and two light chains, with each light-heavy pair cross-linked by disulfide bridges. The Igs are typical globular proteins and are heat labile. This is not normally a problem with whey protein products as the levels are low, but in cases where levels are higher than normal (for example, just post colostrum) they may cause problems. For applications requiring Igs to be biologically active, special low-temperature processing must be used (Bounous *et al*., 1989; Bounous and Gold, 1991).

3.3.4 Chemistry of serum albumin

Bovine serum albumin is found in milk and in whey proteins in varying amounts. It occurs as a result of leakage of the protein from blood serum into the milk at the tight junctions in the cells in the mammary gland. The amount of serum albumin is a function of the health of the cow and in particular the mammary gland; however, levels are usually low. The chemistry of serum albumin is described elsewhere in this volume. For the purposes of this chapter, it can be considered as a minor component of whey protein of high molecular weight (thus well retained in membrane processing), with a large number of disulfide bridges and thus able to react with beta-lactoglobulin (Gezimati *et al*., 1996) and capable of binding a range of low molecular weight materials, which can have a nuisance value.

3.3.5 Chemistry of minor whey proteins

Two proteins of note in the minor whey proteins are lactoferrin and lactoperoxidase. Lactoferrin is considered important because it occurs at much higher levels in human milk, and because it has antibacterial properties. It has also been reported to stimulate processes of bone growth in an *ex vivo* system, although how this might work *in vivo* is unknown. Lactoperoxidase is part of a system that has antibacterial activity. Both of these proteins are present at very low levels in whey protein ingredients, but are available in special preparations from suppliers.

3.4 Technical data

Whey proteins are normally supplied as dry powders (whey protein concentrate or whey protein isolate), which are cream or white coloured, in 25 kg bags. Whey protein isolates are more highly purified than whey protein concentrates, and this will be reflected in price. The more expensive isolates are able to be used in a wider range of applications. Most commercial whey proteins are supplied targeted towards particular applications and a range of supporting literature describing applications is available from suppliers.

3.5 Uses and applications of whey protein ingredients

3.5.1 Nutritional applications

Whey proteins and whey protein hydrolysates are used for specialised nutrition applications, particularly in infant formulae and in specialist enteral nutrition formulations. Hydrolysates have particularly been favoured for enteral nutrition and hypoallergenic infant formulae because the hydrolysis process breaks down possible allergenic structures. Beta-lactoglobulin has been identified as a major potential allergen in cows' milk, and does not occur in human milk.

3.5.2 Infant nutrition

Milk proteins have been used for infant formulae for many years. In the 1990s a lot of attention was paid to the amino acid composition, and particularly the essential amino acid composition of milk protein and its comparison with human milk. A typical comparison is shown in Table 3.3. Mixtures of whey protein and milk protein, such as a mix of 60% whey protein and 40% milk protein, have been widely used as a means of approaching a better balance. The use of whey protein will partly (but not completely) compensate for a low level of tryptophan and cyst(e)ine in milk protein, but results in an excess of threonine and lysine (de Wit, 1998). Lysine is in excess in both milk and whey protein.

Table 3.3 Comparison of essential amino acids in human milk protein, cows' milk protein and whey protein (data from Jost *et al*., 1999). Results are expressed as mg amino acyl/g protein nitrogen. Numbers shown in bold are considered to be beyond the normal range for human milk protein

Amino acid	Human milk	Bovine milk	Bovine whey protein (rennet whey)	Mixture 60:40 (WP:MP)
Threonine	322	279	**462**	**389**
Cyst(e)ine	133	**73**	151	120
Valine	391	380	406	396
Methionine	102	**179**	**140**	**156**
Isoleucine	372	319	400	368
Leucine	671	627	**735**	692
Phenylalanine	275	**330**	214	260
Lysine	466	**540**	**586**	**568**
Histidine	169	185	**114**	**142**
Tryptophan	143	**98**	116	**109**

Table 3.4 Leucine and branched chain amino acids content of food proteins (data from Millward *et al*., 2008)

Protein source	Leucine (g/100g)	Total branched-chain amino acids (g/100g)
Whey protein isolate	14	26
Milk protein	10	21
Egg protein	8.5	20
Muscle protein	8	18
Soy protein isolate	8	18
Wheat protein	7	15

3.5.3 Sports nutrition

Whey protein is particularly suited to sports nutrition because of its high levels of branched-chain amino acids (Table 3.4). Branched-chain amino acids are preferentially metabolised by muscle rather than in the liver. Recent studies have shown that higher blood levels of branched-chain amino acids occurred after consumption of whole whey protein in comparison with hydrolysed whey protein (Farnfield *et al*., 2009). Branched-chain amino acids have been shown to increase protein synthesis and decrease protein breakdown. It has been shown that branched-chain amino acids activate key enzymes involved in protein synthesis in muscle (Blomstrand *et al*., 2006). Branched-chain amino acids have also been found to lead to muscle protein accrual in the elderly (Katsanos *et al*., 2008). In the latter study, the effect was beyond that of equivalent amino acid uptake.

Dipeptides that arise from whey protein digestion may be important. Dipeptides found in whey protein hydrolysates included Ile-Val, Leu-Val, Val-Leu, Ile-Ile, Leu-Ile, and Ile-Leu. All of these were found to stimulate significant glucose uptake rate in L6 myotubes from rats. Ile-Leu was the predominant dipeptide and was shown to stimulate glucose uptake in isolated skeletal muscles from exercise-trained rats (Morifuji *et al*., 2009).

A further aspect of amino acid nutrition that may be important relates to tryptophan uptake in the brain. Tryptophan is a precursor of serotonin, and uptake of tryptophan by the brain for serotonin synthesis is considered to be important for sleep. To be effective, tryptophan must cross the blood–brain barrier. The transport system that takes tryptophan across this barrier is specific only for large neutral amino acids, so the ratio of tryptophan to total large neutral amino acids (Trp:LNAA) will determine its effectiveness. The branched-chain amino acids will compete with tryptophan and limit the rate of tryptophan uptake. This effect is thought to fight fatigue (Blomstrand, 2006). In contrast to this, alpha lactalbumin has a high Trp:LNAA ratio and has been promoted as enhancing sleep (Silber and Schmitt, 2010), so the balance between the major whey proteins is important in any nutritional formulation aimed at altering levels of fatigue or promoting sleep.

Practical formats for sports nutrition using whey proteins include sports drinks and nutrition bars.

3.5.4 Satiety

There is increasing evidence that whey proteins promote satiety (e.g. Luhovyy *et al*., 2007; Dunshea *et al*., 2007). Whey proteins have recently been used in clear "sports water" type drinks that are claimed to promote satiety, supported by some clinical evidence (Poppitt *et al*., 2011), although the evidence for this is, as yet, slight. This application requires a whey protein isolate that is totally soluble in water at mildly acidic pH. There appears to be a growing demand for satiety products as a response to the global obesity problem, and satiety drinks may be an emerging area (Little *et al*., 2009).

3.5.5 Enteral nutrition

Whey proteins are widely used in enteral feeding products, although usually in a hydrolysed format. The relatively high levels of essential amino acids means that low levels of whey protein can be fed to maintain nutrition without overloading total protein input.

3.5.6 Cancer treatment

There have been claims of benefit when feeding whey protein, specifically in a low heat treated (essentially undenatured) form, for cancer patients.

This is proposed to be as a result of elevated levels of glutathione arising from whey protein nutrition (Bounous, 2000; Bounous and Gold, 1991). What is probably more important is the nutritional value for patients undergoing treatment for cancer (Krissansen, 2007).

3.5.7 Functional applications

Whey proteins have functional properties that are widely used in food applications. Particularly important are their ability to stabilise interfaces, giving foaming and emulsifying properties, and their gelling and water-binding properties.

3.5.8 Solubility

For most applications it is necessary for whey proteins to be largely or completely soluble in an aqueous phase of a food or drink. Clear solutions are important for clear drink formulations, such as sport drinks. A recent study on the solubility of whey proteins has found a strong dependence on pH and also dependence on other components in the solution, particularly ionic species (LaClair and Etzel, 2010). Although this study appears to assume denaturation of protein is responsible, these results should probably be reviewed with respect to phase separation being at least in part responsible for cloudiness. Important cations in commercial whey protein products are given in Table 3.5.

3.5.9 Gelation

The gelling and water-binding properties of whey proteins are probably the most important and widely used in food applications. Whey proteins form gels when heated at concentrations above a critical concentration typically around 10% protein. There is a vast literature on the gelation of whey proteins, as commercial whey protein ingredients, as laboratory preparations and as the individual proteins. The key to the gelling performance of

Table 3.5 Typical cation composition of commercial whey protein products

Product type	Sodium (mg/100g)	Calcium (mg/100g)
Acid WPC	31	216
Cheese WPC	340	400
Rennet WPC	247	275
Microfiltered cheese WPI	130	400
Ion exchange WPI	600	64

Source: Fonterra data sheets.

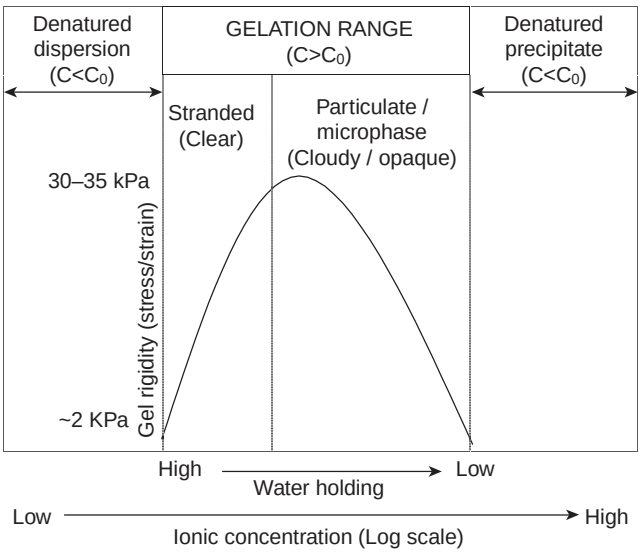


Fig. 3.2 Formation of different gel types as a function of ionic environment, adapted from Foegeding *et al*. (1997). C is the concentration of protein; C_0 is the critical concentration for gel formation.

whey protein is the beta-lactoglobulin and its gelation properties. Gelation is affected by protein concentration, types and concentrations of other proteins and macromolecules, and, most importantly, pH and cations in the medium. Two types of gel have been identified: stranded gels and particulate gels. There is a wide literature on whey protein gels and the two different types. Recent work (Ako *et al*., 2009) has demonstrated that the particulate gels arise from microphase separation of the aqueous phase as described above. Generally, stranded gels arise when there is a clear continuous phase, and are favoured by low pH and relatively low cation concentration. A schematic of the occurrence of stranded and particulate gels is shown in Fig. 3.2, adapted from Foegeding *et al*. (1997). A detailed phase diagram for the different formats of whey proteins when heated at pH 7 is shown in Fig. 3.3a, and pH dependence in Fig. 3.3b. Salt tolerance is lowest close to the isoelectric pH of beta-lactoglobulin (Fig. 3.3b).

The sensory and mechanical properties of the stranded and particulate gels have been described (Gwartney *et al*., 2004; Foegeding, 2006), and are summarised in Table 3.6. Generally stranded gels are firmer, smoother and less sticky than particulate gels.

Whey proteins can also be gelled by treatment with some commercial hydrolytic enzymes, such as the serine protease from *Bacillus lichenformis* (commercial product known as "Alcalase") (Ipsen *et al*., 2001; Doucet *et al*., 2003).

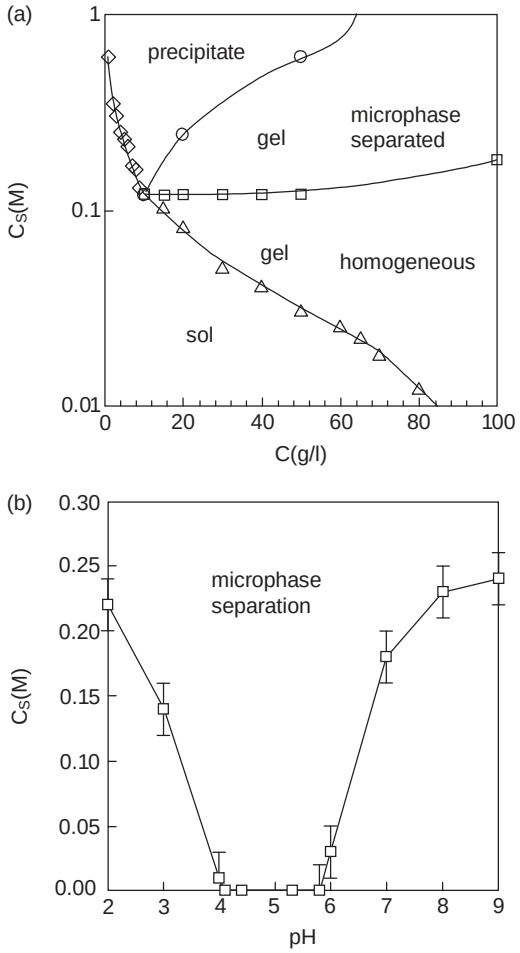


Fig. 3.3 Phase diagrams for whey protein (a) at pH 7 and (b) as a function of pH (from Ako *et al*., 2009 reproduced by permission of the Royal Society of Chemistry). C_S is the concentration of salt in the solution; C is the concentration of whey protein.

3.5.10 Interfacial properties

Foaming and emulsification both depend on the ability of the protein to stabilise an interface between an aqueous phase and an oil or air phase (both of which can be considered to be hydrophobic).

3.5.11 Foaming

Foaming is an important function in the formation of aerated foods such as meringues and angel food cake, for which egg white is the traditional

Table 3.6 Sensory properties of fine-stranded and coarse particulate gels from whey proteins. Data from Foegeding (2006) rounded to nearest unit

Parameter*	Fine-stranded gel	Particulate gel	Description of sensory term
No. of chews	19	15	Number of chews required for swallowing
Smoothness	12	5	Degree to which sample perceived as smooth by tongue
Adhesiveness	1	9	Degree to which the chewed mass sticks to the mouth
Cohesiveness	2	11	Degree to which the chewed mass holds together
Particle shape	9	1	Degree of irregular particle shape
Particle size distribution	3	11	Degree of homogeneity
Particle size	11	1	Size after chews
Crumbliness	1	11	
Moisture release	1	10	Extent of moisture released
Firmness	3	1	Force require to fracture sample with molars
Surface smoothness	12	5	Degree to which the chewed mass surface is smooth

* For details of how the parameters were measured, see Gwartney *et al*. (2004).

foaming protein. Attempts to use whey proteins as egg white substitutes in foaming food applications have been made for many years, with minimal success in many applications – indeed one senior researcher in the area has been known to state that when whey proteins can be used to make angel food cake, he will be ready to retire. Whey proteins in model systems form foams with good properties at typical concentrations around 15% protein. Foaming of whey proteins was systematically examined by Luck *et al*. (2002). The best foams were obtained with whey protein containing higher levels of beta-lactoglobulin. The presence of 0.4M $CaCl_2$ improved foaming properties. Unsuccessful attempts to make angel food cakes using whey proteins have been described and analysed by Foegeding and others (Foegeding *et al*., 2006; Pernell *et al*., 2002; Berry *et al*., 2009). An investigation of the effects of sucrose on the foaming properties of both egg white and whey proteins (Davis and Foegeding, 2007) found that added sucrose improved the foaming properties of egg white foams, leading to increased yield stress and drainage resistance, while it decreased yield stress and drainage resistance for whey protein foams. Interfacial rheology showed that sucrose improved the surface properties (phase angle and interfacial dilational elasticity) of egg white foams and decreased the surface properties of whey protein foams (Davis and Foegeding, 2007). Experiments with purified ovalbumin and beta-lactoglobulin (Davis and

Foegeding, 2007) did not show the same trends, emphasising the difference between proteins on their own and acting in mixtures. A recent study has indicated substantial differences in the performance of whey protein foams in angel food cake formulations when the sucrose is added after foam formation, compared to addition before foam formation (Yang and Foegeding, 2010), but these foams lost structure during cooking. A recent in-depth investigation of the differences between whey protein and egg white protein in angel food cake (Berry *et al*., 2009) indicates that the main difference between the two types of protein is a combination of bubble disproportionation in the foam and a loss of stability during heating in the whey protein foams.

3.5.12 Emulsification

The emulsifying properties of dairy proteins in model systems have been described by a number of authors (Euston and Hirst, 2000; Singh and Ye, 2009). Whey proteins can act as effective emulsifiers at low concentrations (0.5%), and form thin interfacial layers, in contrast to those produced by protein aggregates such as casein micelles. Whey protein concentrates were shown to be good emulsifiers in simple oil-in-water emulsion systems, comparable with sodium caseinate and casein. In model coffee whiteners, however, differences were apparent and the stability of whey protein emulsions was less than that of emulsions stabilised by aggregated proteins (Euston and Hirst, 2000). There is a plethora of literature describing the effects of other ingredients on emulsions using whey proteins. Recent examples include calcium (Ye and Singh, 2000), pectin (Mishra *et al*., 2001), carboxymethyl cellulose (Damianou and Kiosseoglou, 2006; Koupantsis and Kiosseoglou, 2009), xanthan (Benichou *et al*., 2007), flax seed gums (Khalloufi *et al*., 2009), and a range of carbohydrates (Herceg *et al*., 2007; Euston *et al*., 2002). In addition to this, modification of whey protein by denaturation (Dissanayake and Vasiljevic, 2009; Euston *et al*., 2001a), partial hydrolysis (Euston *et al*., 2001b) and conjugation with sugars (Lillard *et al*., 2009) have all been found to change emulsifying properties.

3.5.13 Interaction with carbohydrates

In many food applications, whey proteins are used together with sugars and/or polysaccharides. The effects of sucrose on foaming have already been discussed. Interactions with soluble carbohydrates and other hydrocolloids can lead to phase separation, with or without loss of solubility through depletion flocculation, or to complex coacervation, and these changes are likely to be the cause of discontinuities in functional performance. The interaction of milk proteins with carbohydrates has been recently reviewed by Goh *et al*. (2009). The interaction between whey proteins and some polysaccharides is summarised in Table 3.7. This list is not exhaustive,

Table 3.7 Some examples of phase behaviour of whey proteins with polysaccharides – after Goh *et al.* (2009)

Polysaccharide	Interaction type	Reference
Gum arabic	Complex formation; complex coacervation	(Weinbreck *et al*., 2003)
Pectin	Complex formation, no phase separation	(Mishra *et al*., 2001)
Xanthan gum	Co-solubility	(Hemar *et al*., 2001)
Kappa-carrageenan	Destabilisation of emulsions	(Singh *et al*., 2003)
Lambda carrageenan	Complex formation, precipitation	(Weinbreck *et al*., 2004)
Galactomannan (Locust bean gum)	Biphasic gel	(Monteiro *et al*., 2005)

and it will be necessary to consult the wider literature and perform tests before using whey protein when developing food formulations with soluble carbohydrates.

3.6 Whey protein hydrolysates

Whey protein hydrolysates are widely used for paediatric nutrition and for sports nutrition. Some hydrolysates also have desirable functional properties.

3.6.1 Manufacture of hydrolysates

Hydrolysates are normally manufactured by incubating with proteolytic enzymes at elevated temperature (typically 37–40 °C). The degree of hydrolysis (DH) is an important characteristic, and is defined as the percentage of peptide bonds cleaved (Adler-Nissen, 1976). DH is carefully monitored and enzyme action is stopped when the desired DH is reached, usually by heating. The mixture of enzymes used and exact processing conditions will vary from one manufacturer to another and this is usually proprietary information. Whey protein hydrolysates are often described by a combination of DH and some description of the range of molecular weight of the component peptides. The latter is often shown as a size-exclusion chromatogram of the product. Desired targets in the manufacture of whey protein hydrolysates include:

- avoidance of large residual peptides
- avoidance of bitter flavours (usually caused by peptides with high levels of hydrophobic amino acid residues)
- close control of the spread of molecular weight of peptides.

Following hydrolysis, the hydrolysates are usually spray dried and are supplied as a spray-dried powder.

3.6.2 Flavour of whey protein hydrolysates

When whey proteins are hydrolysed and subsequently processed, a range of off flavours can develop. In particular, high degrees of hydrolysis can lead to hydrophobic peptides that are characterised by bitter flavours. In addition, cooked, malty and potato flavours have been reported (Leksrisompong *et al*., 2010). When using whey protein hydrolysates, it is important to ensure that any off flavours are minimised or suitably masked.

3.6.3 Nutritional uses of whey protein hydrolysates

The most important use of whey protein hydrolysates is in paediatric nutrition. A high level of whey protein is desirable in infant formula, to better reflect the amino acid composition of human milk (see Table 3.3); however, some whey proteins, notably beta-lactoglobulin (which is not present in human milk) are considered to be potential allergens. Hypoallergenic infant formulae are generally based on the use of milk protein and whey protein hydrolysates. Hydrolysis of whey proteins for paediatric use should result in hydrolysis of the beta-lactoglobulin so that there are no remaining peptides large enough to contain epitopes. This means a relatively high DH, but DH must be limited as too high a DH will result in a high osmotic load, which can cause gastrointestinal upset, and high DH can result in bitterness.

Hydrolysed whey protein is also used in sports nutrition. Use of whey proteins for sports nutrition is described earlier in this chapter. The benefit of hydrolysis is thought by some to be that the protein is "pre-digested" and can result in more rapid uptake of amino acids, although there is little evidence to support this view (Farnfield *et al*., 2009).

3.6.4 Functional properties of whey protein hydrolysates

When whey proteins are hydrolysed, functional properties are altered. In particular, hydrolysis using the commercial protease from *Bacillus lichenformis* (for example "Alcalase") can lead to formation of aggregates and gels (Creusot and Gruppen, 2007; Spellman *et al*., 2005; Doucet *et al*., 2003). This is believed to be due to a glutamyl endopeptidase found in the enzyme mixture (Spellman *et al*., 2005).

As well as gelation, hydrolysis may affect solubility, foaming and emulsifying properties. These functionalities can vary depending on the whey source, the enzyme(s) used for the hydrolysis, DH and the conditions of processing during and subsequent to hydrolysis. It is thus important to consult manufacturers' specifications and to test samples before using these ingredients for product development.

3.7 Regulatory status

Whey proteins form part of milk, a food that has been consumed from time immemorial and recognised as safe. Whey protein concentrates and whey protein isolates have GRAS (generally recognised as safe) status from the American Food and Drug Administration (FDA) under regulation 21 CFR 184.1979c, subject to being manufactured from pasteurised milk under good manufacturing practice. Whey protein products are similarly recognised in most jurisdictions.

Whey protein products are subject to import restrictions and customs tariffs in many jurisdictions, and ingredients with less than 80% protein are not permitted to be imported or subject to additional quota and tariff restrictions in many countries and regions.

3.8 Future trends

3.8.1 Threat of plant-derived replacements

Milk is expensive in terms of resource inputs. Milk is an animal product, and to produce it requires the cow to eat vegetable material that is already in a nutritional format. However, milk is the most efficiently produced of the animal-produced foods – largely because the animal itself is not consumed. It has been estimated that production of milk protein in the USA requires 585 kJ/kg, an energy efficiency of 30:1 (i.e., a requirement of 30 kJ input energy for every kJ of protein energy). In contrast, the total energy input per kg of production of corn or soy protein in the USA calculates out to 58 kJ/kg (data calculated from Pimentel and Pimentel, 1979). We have estimated that production of 1 litre of milk under New Zealand farming conditions requires 1400 litres of water (Rowney, Boland and Golding, unpublished), corresponding to about 40,000 l water per kg protein. The major proportion of these figures is for the on-farm production costs, and estimates of use in processing are negligible by comparison. The consequence of this is that whey proteins are, and will continue to be, expensive ingredients, and plant-based substitutes will continue to be developed that will replace whey proteins in some applications. This is already the case with many functional proteins, although differences in functional performance and requirements for labelling claims still make whey proteins a preferred option for many foods. The acceptance of genetically engineered proteins will allow the improvement of the nutritional value of plant proteins, currently a weakness.

3.8.2 Potential of single variant whey proteins

The whey proteins exist in several genetic variants, and particularly beta lactoglobulin A and B have different functional properties (Foegeding

et al., 1999; Euston *et al*., 1997). Although it is unlikely that herds will ever be bred for single variants for the purpose of producing functional proteins, it is possible that breeding for other purposes may lead to variant selection – for example beta-lactoglobulin B variant produces more casein in the milk, leading to higher cheese yields (Boland and Hill, 2001).

3.8.3 Possible new formats and applications

A range of modified whey proteins is currently being explored with modified functionality. None of these is widely available as an ingredient at the time of writing, but some of these might be expected to be seen in the future.

3.8.4 Fibrils and nanotubes

Whey proteins, particularly alpha lactalbumin and beta-lactoglobulin, are known to produce nanostructures when incubated at acid pH (Graveland-Bikker and de Kruif, 2006). Alpha lactalbumin forms nanotubes as a result of limited enzyme hydrolysis in the presence of calcium. Beta-lactoglobulin can form a variety of fibrils at nano scale. It is believed that limited acid hydrolysis may play a part in this structure formation. The process of formation of these fibrils is being intensively studied and is likely to lead to novel specialised whey protein functional ingredients, particularly for gelation and viscosity (Akkermans *et al*., 2008; Loveday *et al*., 2010).

3.8.5 High pressure processed protein

Whey proteins are susceptible to conformational modification by high hydrostatic pressure. In particular, beta lactoglobulin denatures under relatively mild pressure conditions. Developments of high pressure processing equipment for the food industry over the past 20 years have made commercial high pressure processing feasible. There has been extensive investigation into whey protein with modified properties due to high pressure processing (e.g. Lim *et al*., 2008; Patel *et al*., 2005; and reviewed by Considine *et al*., 2007 and Patel and Creamer, 2009). It seems likely that pressure-treated whey protein ingredients will emerge over the next decade.

3.8.6 Cold-gelling whey protein

So-called cold-gelling whey proteins can be produced by limited heat treatment of whey protein at low concentrations (Bryant and McClements, 2000). The solutions so produced will gel in response to increased salt concentrations. Whether commercial cold-gelling whey proteins will

become widely available (or whether there is a demand for it) remains to be seen.

3.8.7 Organic whey proteins

There is increasing production of organic foods globally, and substantial quantities of organic milk are being produced. Fonterra was quoted as having 70 organic supply farms in 2007, expected to grow to 200 by 2013 (Source: New Zealand Department of Trade and Enterprise website: http://www.marketnewzealand.com/common/files/Dairy-industry-in-New-Zealand.pdf). Production of organic cheese and whey proteins seems an obvious consequence. It is likely that organic production will have little effect on the properties of whey protein as a functional or nutritional ingredient; however, organic label claims may become possible.

3.8.8 Carbohydrate conjugates

It is well recognised that conjugation of whey proteins with sugars has the potential to change their properties. The Maillard reaction is the process usually used to develop such conjugates, and although this leads to loss of bioavailable lysine, functional benefits can outweigh this. An investigation comparing commercial whey protein concentrate with whey protein concentrate that had been heated to conjugate with the lactose naturally present, or conjugated with dextrans that had been introduced, showed increased viscosity and enhanced emulsifying performance (Lillard *et al.*, 2009). Conjugates with maltodextrin have also been characterised (Akhtar and Dickinson, 2007). This may pave the way for a new range of whey protein-based specialised ingredients.

3.9 Sources of further information and advice

There is a very extensive literature dealing with the use of whey proteins as food ingredients, a small proportion of which is referenced in this chapter. In particular, the review articles are recommended. Understanding of the nutritional and functional performance of whey proteins is still developing and a watch on the literature is recommended. Specific sources of further information include:

- *Food Hydrocolloids*: this periodical has regular research papers concerning the functional properties of dairy proteins.
- *Milk Proteins: from expression to food* (Ed Thompson, A., Boland, M. and Singh, H.; Academic Press, 2009): A recent book with a range of chapters following a spectrum of reviews of milk proteins from genetic and production issues through to chemistry, processing, functionality and use in foods.

3.10 Acknowledgements

I would like to thank Allen Foegeding and Harjinder Singh for assistance with this chapter, and Fonterra Cooperative Group Ltd for access to unpublished material. This work was supported in part by the Foundation for Research Science and Technology under programme MAUX 0703.

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4

Meat protein ingredients

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Abstract: This chapter discusses meat protein ingredients, a class of high-protein products that are derived from either meat animal by-products or lean tissue components and that are used primarily as ingredients in meat and other food products. Specifically, the chapter reviews protein ingredients derived from lean meat tissues, connective tissues and blood. The discussions are centered on the obtainment, functional properties, food applications and current regulatory aspects of each.

Key words: meat protein ingredients, lean tissue protein ingredients, connective tissue protein ingredients, hydrolysates and flavors, blood protein ingredients.

4.1 Introduction

The meat protein ingredients discussed in this chapter are a class of high-protein products that are derived from either meat animal by-products or lean tissue components (Table 4.1), and that are used primarily as ingredients in meat and other food products. This definition excludes skeletal muscle in its original, unmodified, physical and chemical structural forms and, therefore, its uses as a primary raw material source for processed meat products fall outside the scope of this discussion. Regardless of their actual application, however, these ingredients may be considered either meat or non-meat by applicable regulations, depending on their specific source or on their extraction and isolation procedures. As their regulatory classification may affect how they are utilized, it is important to have a clear and adequate understanding of their status in both practical and regulatory terms.

4.1.1 Meat and its derivatives

In a practical, pragmatic, sense, meat can be defined as 'the edible postmortem component originating from live animals,' particularly 'domesticated

Table 4.1 Major sources and types of meat protein ingredients

Source	Ingredients
Lean tissue	Finely textured meat/poultry
	AMR (advanced meat recovery) meat/poultry
	Mechanically separated meat/poultry
	Meat protein isolates
Bone	Gelatin (type B)
	Edible bone collagen (ossein)
	Bone collagen hydrolysates (stocks and broths)
Pig skin	Gelatin (type A)
	Stocks and broths
Beef hides	Gelatin (type B)
Poultry skin (chicken, turkey)	Concentrated collagen
	Stocks and broths
Collagen-rich tissues	Concentrated collagen
	Collagen hydrolysates
Blood	Blood plasma (liquid, frozen, dried)
	Whole blood (liquid and dried)
	Red cell protein (decolorized)
	Plasma transglutaminase

cattle, hogs, sheep, goats and poultry, as well as wildlife such as deer, rabbit and fish' (Kauffman, 2001). Although organ meats such as hearts or livers are sometimes considered meat, the term is often restricted to the edible skeletal muscle tissue of mammalian species (primarily bovine and porcine), poultry, and seafood. It is in this context that the term is utilized in this chapter.

Regulations, on the other hand, define meat in a much more specific and restrictive way and, to complicate matters, these definitions oftentimes vary by country. United States regulations define meat as:

> The part of the muscle of any cattle, sheep, swine, or goats which is skeletal or which is found in the tongue, diaphragm, heart, or esophagus, with or without the accompanying and overlying fat, and the portions of bone (in bone-in product such as T-bone or porterhouse steak), skin, sinew, nerve, and blood vessels which normally accompany the muscle tissue and that are not separated from it in the process of dressing. (Code of Federal Regulations [CFR], 2010a)

Also included in the definition of meat are materials derived from advanced meat/bone separation and meat recovery (AMR) systems, which will be discussed later.

Materials derived from both AMR and traditional mechanical separation systems have been and are presently being utilized as total or partial replacements for unmodified, intact skeletal muscle in meat product formulations. However, given that they can also be used as additives and that their

physical structure represents a modification of that of the starting raw materials, they fall within the scope of this chapter and will be discussed in more detail later.

In the US, poultry tissues are regulated separately from meat. Poultry is defined as 'any domesticated bird (chickens, turkeys, ducks, geese, guineas, ratites, or squabs, also termed young pigeons from one to about thirty days of age), whether live or dead' (CFR, 2010i), whereas poultry *product* is defined as 'any poultry carcass or part thereof; or any product which is made wholly or in part from any poultry carcass or part thereof. . . . Except where the context requires otherwise . . . this term is limited to articles capable of use as human food' (CFR, 2007).

In the European Union, meat is defined as (European Parliament and Council, 2007):

> [s]keletal muscles of mammalian and bird species recognized as fit for human consumption with naturally included or adherent tissue, where the total fat and connective tissue content does not exceed the values indicated in [Table 4.2] and where the meat constitutes an ingredient of another foodstuff. The products covered by the Community definition of 'mechanically recovered meat' are excluded from this definition.

This definition, contrary to USDA's, does include poultry. However, it does exclude mechanically recovered meat, which must, therefore, be labeled separately and cannot be considered part of a product's meat content (European Commission, Health and Consumer Protection Directorate-General, 2001). Also excluded from the definition are '[o]ther meat-related ingredients derived from meat protein, fat and connective tissue and which have undergone a treatment such as purification (e.g. gelatine, collagen fibre, refined fats, . . .), hydrolysation (e.g. protein hydrolysates, . . .), extraction (e.g. meat extracts, bouillons, .) . . .' (CLITRAVI, 2002), which includes many of the ingredients discussed in this chapter. It is important

Table 4.2 Maximum fat connective tissue contents for ingredients designated by the term 'meat'

Species	Fat (%)	Connective tissue[1] (%)
Mammals (other than rabbits and porcines) and mixtures of species with mammals predominating	25	25
Porcines	30	25
Birds and rabbits	25	25

[1] The connective tissue content is calculated on the basis of the ratio between collagen content and meat protein content. The collagen content means the hydroxyproline content multiplied by a factor of 8.

to understand that in the EU the term 'animal by-product(s),' or ABP, refers exclusively to products of animal origin that are not intended for human consumption (Commission of the European Communities, 2006; European Parliament and Council, 2002; European Union, 2007). The EU general rules regarding meat by-products for human consumption, including definitions, can be found in Regulation (EC) No. 853/2004 (European Parliament and Council, 2004).

In the US, meat by-products themselves are classified by the USDA as 'Group 1 Protein-Contributing Ingredients.' However, proteinaceous ingredients derived from them by 'hydrolysis, extraction, concentration, or drying' are classified as 'Group 2 Protein-Contributing Ingredients' (CFR, 2010c; United States Department of Agriculture, Food Safety and Inspection Service [USDA-FSIS], 1995b), and must be considered 'non-meat protein' when formulating, as when calculating protein fat-free (PFF) in cured pork products (CFR, 2010h) and added water in cooked sausage products (CFR, 2010c).

This chapter focuses on those protein ingredients derived from meat animals that have been found to be technically viable for the formulation of food products. Although they are termed meat protein ingredients, it must be pointed out that many of them do not comply with the definition of meat. The reader is therefore encouraged to consult local regulations prior to their commercial application.

4.2 Sources of meat protein ingredients

There are numerous potential sources of meat protein ingredients (Table 4.1), some of which are presently more commercially feasible than others. The most commonly used meat protein ingredient over the years has been gelatin, although there are a number of other meat protein ingredients with different functional properties, many of which have been available in some form for some time. However, new advances in protein chemistry, as well as in extraction and purification technologies, have resulted in products with novel and improved functional properties that continue to challenge the limits of what is considered technically and commercially feasible.

4.3 Lean tissue protein ingredients

After the higher-valued meat cuts and trimmings have been harvested from a meat animal, significant amounts of lean tissues still remain on the carcass, either attached to bones or to low-value fatty trimmings. It has been estimated that approximately 30% of the weight of manually-trimmed bones of red meat carcasses is edible meat, which could amount to 6–10 kg in a beef carcass and 1–2 kg in a pork carcass (Ockerman and Hansen, 2000).

Table 4.3 World production of beef in 2008, by number of animals slaughtered. Top ten producing countries

Rank	Country	Number of head	% of world production
1	China	43 575 429	14.62
2	Brazil	39 795 000	13.35
3	United States of America	34 514 400	11.58
4	Argentina	13 500 000	4.53
5	India	12 216 000	4.10
6	Russian Federation	9 598 047	3.22
7	Australia	9 100 000	3.05
8	Mexico	8 074 451	2.71
9	France	5 008 900	1.68
10	Colombia	4 250 000	1.43
	World total	297 979 830	100.00

Source: FAO Statistical Database (FAO, 2010)

Table 4.4 World production of pork in 2008, by number of animals slaughtered. Top ten producing countries

Rank	Country	Number of head	% of world production
1	China	620 766 550	47.28
2	United States of America	112 000 000	8.53
3	Germany	54 847 564	4.18
4	Spain	41 305 540	3.15
5	Viet Nam	37 000 000	2.82
6	Brazil	33 315 000	2.54
7	France	25 291 200	1.93
8	Russian Federation	24 068 236	1.83
9	Philippines	23 805 040	1.81
10	Poland	22 359 600	1.70
	World total	1 312 945 834	100.00

Source: FAO Statistical Database (FAO, 2010)

Given that – according to statistics from the Food and Agriculture Organization of the United Nations (FAO) – 298 million beef cattle and 1313 million swine were slaughtered commercially in the world in 2008 (FAO, 2010; Tables 4.3–4.6), this amounts to 1788–2980 million kg of beef and 1313–2626 million kg of pork per year, which represents a significant amount of edible meat. Recovery and utilization of the meat that remains attached to carcass bones and low-value trimmings, therefore, increases the economic value of these raw materials and provides additional sources of high-quality protein for use as human food, resulting in a more efficient utilization of agricultural resources.

Table 4.5 World production of chicken in 2008, by number of animals slaughtered. Top ten producing countries

Rank	Country	Number of head (× 1000)	% of world production
1	United States of America	9 075 261	17.16
2	China	7 759 196	14.67
3	Brazil	5 465 780	10.33
4	India	2 615 000	4.94
5	Indonesia	1 904 000	3.60
6	Mexico	1 513 341	2.86
7	Russian Federation	1 320 232	2.50
8	Iran	1 186 000	2.24
9	Thailand	900 166	1.70
10	United Kingdom	822 753	1.56
	World total	52 887 284	100.00

Source: FAO Statistical Database (FAO, 2010)

Table 4.6 World production of turkey in 2008, by number of animals slaughtered. Top ten producing countries

Rank	Country	Number of head (× 1000)	% of world production
1	United States of America	260 000	38.89
2	Brazil	68 500	10.25
3	France	62 900	9.41
4	Germany	50 000	7.48
5	Italy	29 959	4.48
6	Chile	27 000	4.04
7	Canada	22 849	3.42
8	Israel	15 000	2.24
9	United Kingdom	14 925	2.23
10	Poland	12 000	1.79
	World total	668 616	100.00

Source: FAO Statistical Database (FAO, 2010)

4.3.1 Mechanically separated meat

Obtainment and manufacture

Mechanically separated meat (MSM) or poultry (MSP) result from a process by which edible muscle tissue is recovered from bones by forcing the latter through very small orifices. The process, which is generally used when there is no other economically feasible way to recover edible tissue from bones, typically starts by grinding the bones through a 1.3–3 cm plate prior to feeding them to a deboning machine. There are three primary types of deboning systems (Field, 1988). One uses a rotating auger inside a

cylinder-shaped sieve with orifices approximately 0.5 mm in diameter. Bone is retained on the inside of the cylinder and conveyed out to the end, while the meat passes through the orifices. In a second type of system, bones are squeezed between a rubber belt and a perforated steel drum, with the meat passing through the drum perforations and most of the bone remaining on the outside. A third type of deboning machine is a batch type of system, where bones are pressed against a stationary slotted surface (Field, 1988; Ockerman and Hansen, 2000). Other types of mechanical meat deboners have been developed and commercialized. Regardless of the deboning system used, the resulting edible material (MSM or MSP), has the consistency of a thick paste or batter and is, therefore, suitable mostly for use in products where muscle fiber structure is either not desired or unimportant, such as comminuted sausage products (e.g. frankfurter, bologna).

Functional properties

The chemical composition of MSM/MSP is highly dependent on factors such as animal age, types of bones used, bone/meat ratio, skin content, deboner settings (e.g., orifice size, pressure) and even bone temperature and conditioning prior to deboning. This makes it almost impossible to make generalizations regarding its composition. However, mechanically separated meats do generally contain higher levels of fat, ash and calcium, and lower levels of moisture and protein, when compared to their hand-deboned counterparts (Ockerman and Hansen, 2000). MSM/MSP also contains more bone marrow and powdered bone than hand-deboned meat, which has led regulatory authorities to include such factors as iron and calcium content and bone particle size in the material's legal standards.

During the manufacture of MSM/MSP, the functionality of the resulting material may be affected by some of the above-mentioned factors. High-yield settings, for example, may affect functionality by increasing temperature and causing protein denaturation. In addition, the combination of unsaturated fat from meat, skin and bone marrow, extreme particle size reduction, incorporation of air, contact with metal and elevated processing temperatures can contribute to the relatively rapid oxidative deterioration of the material, resulting in rancid flavors and off-colors (Field, 1988; Froning and McKee, 2001). The oxidative stability of the material may also vary by species, with mechanically separated turkey being particularly unstable (Dimick *et al.*, 1972). To mitigate the effects of oxidative rancidity, the use of antioxidants (when necessary) as well as proper handling and temperature control techniques have been utilized successfully.

Uses and applications

MSM and MSP have been used successfully in the meat industry worldwide for a number of years. Initially used strictly as replacements for higher-cost meat trimmings, particularly in comminuted products, e.g. frankfurters and bologna, they have since become primary raw materials in their own right.

Regulatory aspects

In the US, MSM is excluded from the definition of meat and, when used, must be labeled as 'mechanically separated (pork, veal, or lamb)' (USDA-FSIS, 2006b). Mechanically separated beef was declared inedible and prohibited for use as human food in early 2004, out of concerns related to bovine spongiform encephalopathy (BSE) (CFR, 2010e; USDA-FSIS, 2004). MSP, on the other hand, may be used without limit in the formulation of poultry products (CFR, 2010j).

In the EU, the production of MSM from ruminant bones is regulated based on the BSE status category assigned to the originating country or region, as described in Article 9 of Regulation (EC) No. 999/2001 (European Parliament and Council, 2001). Generally, MSM cannot be produced from any ruminant material from category 5 countries or regions, or bones of the head and vertebral columns of bovine, ovine and caprine animals from category 2, 3, 4 or 5 countries or regions. Permissible MSM (practically speaking, that from swine or poultry) is defined in Annex I of Regulation (EC) No. 853/2004 (European Parliament and Council, 2004) as 'the product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fibre structure,' and must comply with the requirements of that legislation. It is not considered meat, and must therefore be labeled separately and not be counted as part of a product's meat content (European Commission, Health and Consumer Protection Directorate-General, 2001) (see Section 4.1.1).

4.3.2 Meat from advanced meat recovery (AMR) systems

Obtainment and manufacture

So-called advanced meat recovery (AMR) is a technology that removes muscle and other edible tissues from the bones of animal carcasses without the incorporation of bone. This is accomplished by machines that shave, scrape or press the muscle tissues away from the bone in such a way that the resulting material's muscle fiber structure is retained. As a result, the material has an appearance, texture and composition comparable to hand-deboned ground meat trimmings. Other terms used to describe this material include desinewed meat, Baader meat, or 3 mm meat, among others. Differences in the types of mechanical equipment utilized, as well as in the types of bones fed into them, may affect processing yields as well as the compositional properties of the resulting material (Field, 1988; Hasiak and Marks, 1997).

Functional properties

AMR meat has been shown to be higher in total pigment, iron and calcium and lower in collagen than hand-deboned meat. However, it is structurally similar to hand-deboned trimmings, and has been found to be a suitable

replacement at levels of 15% or higher (Calhoun *et al*., 1999). Still, it must be borne in mind that the functional properties of the material are a function of the way in which it is obtained. Therefore, it is important to understand what these properties are and account for them during product formulation.

Uses and applications

AMR meat is typically used as a replacement for hand-deboned trimmings in products such as hamburger and sausage. In recent years the more widespread use of AMR beef has been hindered by concerns related to bovine spongiform encephalopathy (BSE), but both US and European food safety authorities have taken aggressive measures to deal with this risk (see Regulatory aspects below).

Regulatory aspects

In the US, material obtained from AMR systems is defined as meat as long as the machinery does not 'grind, crush, or pulverize bones to remove edible meat tissue' and the 'bones . . . emerge essentially intact' (USDA-FSIS, 2006b). More specifically, 9 CFR § 318.24 (CFR, 2010d) mandates that (i) the materials must not be derived from the skull or vertebral column bones of cattle 30 months of age or older, (ii) the recovery system must not incorporate bone solids or bone marrow in excess of specified requirements, as measured by calcium and added iron contents not exceeding 130 mg and 3.5 mg per 100 g, respectively, and (iii) the materials must be devoid of tissues of brain, trigeminal ganglia, spinal cord, or dorsal root ganglia (DRG), regardless of animal age or type of bone utilized. Products that do not comply with the calcium and added iron requirements of this section may use a common or usual name that is not false or misleading, with the exception being that the name 'Mechanically Separated (Beef)' may not be used. If a product does not meet the requirements of this section but does meet those of 9 CFR § 319.5 for 'Mechanically Separated (Species)' (CFR, 2010e) it may be labeled and used as such. Figure 4.1 shows a decision matrix based on these requirements. Because it meets the definition of meat, there are no limits regarding its usage level in meat products.

In the EU, AMR meat falls outside the definition of mechanically separated meat (MSM) of Regulation (EC) No. 853/2004, because the process does not result in the 'loss or modification of the muscle fiber structure.' It has been argued that it falls within the definition of a meat preparation, as defined in Annex I, paragraph 1.15, of Regulation (EC) No. 853/2004, which includes fresh meat 'that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fiber structure of the meat and thus to eliminate the characteristics of fresh meat' (Food Standards Agency, 2009). There is currently no standard that quantifies the degree of muscle fiber modification that can be used to clearly define when

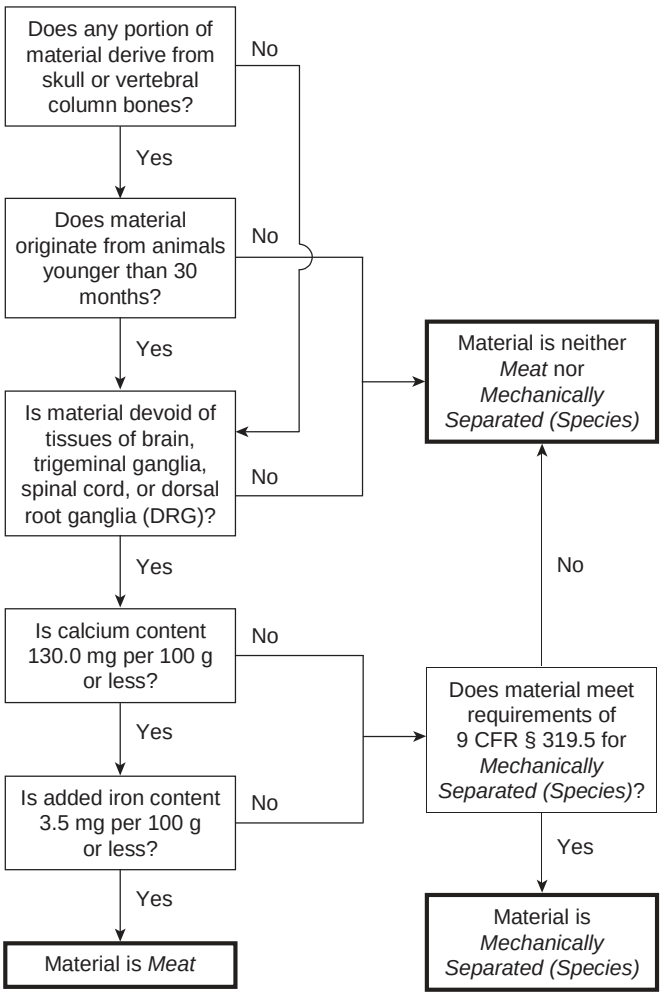


Fig. 4.1 Decision matrix for determining the regulatory status of material from AMR systems in the US (based on CFR, 2010d).

a material falls outside MSM standards, although proposals have been presented to deal with this situation (Sifre *et al*., 2009).

4.3.3 Finely textured tissue

Obtainment and manufacture

Finely textured tissue (FTT) is a lean (15% fat) material typically obtained by the low-temperature separation of high-fat (>60–65% fat) meat or poultry trimmings. During the process, the trimmings are heated to a temperature in

the range of 32 to 48°C – much below that required for the denaturation of meat proteins – in order to facilitate the separation of fat from lean, which is accomplished through centrifugation. Variations of this basic process yield products with slightly different compositional and functional properties and have led to the publication of numerous patents. Another way to alter the composition of the finished materials is to feed the system raw materials from different sources and of varying chemical composition.

Functional properties

It has been reported that the functional properties of FTT are generally inferior to those of lean muscle, especially when used in comminuted meat products. In one study, lean finely textured tissue (LFTT) from beef (LFTB) and pork (LFTP) were used in frankfurter formulations and found to yield an acceptable finished product, but only with an increase in the level of sodium chloride or the addition of kappa-carrageenan and sodium tripolyphosphate (He and Sebranek, 1996b). In a separate study, the same researchers reported lower gel strength and higher water loss in protein gels made from LFTB and LFTP, as compared to protein gels made from beef chuck and pork shoulder, respectively. This lower functionality was attributed to the observation that LFTB and LFTP had a lower content of myosin and actin and a higher content of collagen than their lean meat counterparts (He and Sebranek, 1996a).

Uses and applications

FTT materials are commonly utilized in commercial ground beef and hamburger, at inclusion levels of up to approx. 30%. Their use in comminuted meat products is likely limited to a lower inclusion level due to their inferior functionality, and is dependent on the type of product, the specific formulation and the presence and amounts of other ingredients. It is conceivable that they could be used at higher levels in products that do not rely too much on the meat proteins' functional properties.

Regulatory aspects

In the US, when the material is derived from fatty trimmings containing less than 12% lean, it is referred to as Partially Defatted (Beef or Pork) Fatty Tissue (CFR, 2010f, 2010g) and may be used in products where meat byproducts are permitted, such as nonspecific loaves, beef patties, frankfurters with byproducts, bologna with variety meats, imitation sausage, potted meat food product, sauces, or gravies (USDA-FSIS, 2005). It is allowed in 'Beef Patty Mix' but not 'Ground Beef,' and must always be declared on the ingredient statement. Material derived from tissue containing at least 12% lean is referred to as Finely Textured (Beef or Pork) or Partially Defatted Chopped (Beef or Pork). It is not permitted in hamburger, ground or chopped beef, but it is permitted in other products, as specified in the USDA Food Standards and Labeling Policy Book (USDA-FSIS, 2005).

4.4 Connective tissue protein ingredients

Connective tissue (CT) is the primary component of the extracellular matrix of skeletal muscle and forms the network that surrounds and holds muscle fibers in place. Connective tissues include bone, cartilage, skin, vascular tissues and basement membranes (e.g., endomysium) and are made up of protein fibers, ground substance (loose material composed of glycoproteins, carbohydrates, lipids and water) and other cell types. The major component of CT, up to 95% in some cases, is the fibrous protein collagen (Bandman, 1987; Tarté and Amundson, 2006).

4.4.1 Collagen

Collagen is a family of insoluble fibrous proteins found in all multicellular organisms. One of the most abundant proteins in nature, it is the most abundant protein in mammals, accounting for about 25–30% of total body protein (Bailey and Light, 1989), 1–2% of bovine skeletal muscle and 4–6% of high-connective tissue muscles (Whiting, 1989). It is a major component of skin, bone, cartilage, tendon, blood vessels, basement membrane (endomysium), and teeth (Bandman, 1987; Stryer, 1988). Collagen is a rod-shaped molecule approximately 1.5 nm in diameter and 300 nm in length. Its basic subunit is called tropocollagen (mol wt 300 kDa), and consists of three helical polypeptide α-chains (α1, α2, and α3) coiled around one another into a triple-stranded superhelix that is stabilized by hydrogen bonds. Various collagen phenotypes, 19 of which have been identified, arise from variations in the composition of the tropocollagen α-chains (Bailey and Paul, 1998; McCormick and Phillips, 1999). Of these, the most abundant in meat are the fibrous types I, III and V, and the non-fibrous type IV. Type I collagen predominates in bone, tendon, skin and the epimysium, types I and III in the perimysium, and types III, IV and V in the endomysium (Sims and Bailey, 1981). The molecule's C- and N-termini make up about 2–3% of the molecule from either end and consist of small, non-helical regions called telopeptides.

Collagen has a very unique amino acid composition and sequence. Compositionally, it is approximately 33% glycine, 12% proline, 11% hydroxyproline, and 11% alanine; it is also devoid of tryptophan, and contains the unusual amino acids 3-hydroxyproline, 4-hydroxyproline, and 5-hydroxylysine (Bechtel, 1986). A collagen chain's sequence has three amino acid residues per helical turn; every third amino acid is a glycine residue which, being small, occupies the helice's interior positions.

Obtainment and manufacture

Collagen for use in foods has been obtained from bone (as bone collagen extract), beef hides, pork skins, and skeletal muscle connective tissue (Gillett, 1987). Skeletal muscle tissue collagen can be concentrated by

mechanical desinewing or extracted by low-temperature rendering followed by extrusion, dehydration, grinding, flaking, or milling (Gillett, 1987; Jobling, 1994; Prabhu and Doerscher, 2000; Prabhu and Hull, 2001). In either form, it has been shown to significantly affect the processing characteristics and organoleptic attributes of the meat products in which it is incorporated.

Functional properties

Collagen's functionality depends on various factors, e.g. animal species and age, anatomical source, and extraction conditions. Manipulation and control of these factors, therefore, allows for selective manipulation of the collagen's functional properties. The potential use of collagen as a functional additive in meat products dates back to at least the late 1960s (Elias *et al*., 1970). Since then, much research has focused on ways to extract it from various sources (species, anatomical locations) and by various technological means, as well as on its application in various types of meat products.

The physical extraction and/or concentration of collagen usually involves particle size reduction of collagen – or high-collagen materials – by cutting, grinding, flaking, milling, or a combination of these. Frequently, dehydration and/or freezing steps are incorporated into the process. Some of these approaches have been well documented (Eilert *et al*., 1993; Elias *et al*., 1970).

The development of low-temperature rendering systems has resulted in the commercialization of functional concentrated collagen ingredients. This process, which may vary slightly by manufacturer, involves the addition of steam and hot water to soft materials such as lean and fatty trimmings and pig skins. A decanter centrifuge is then used to separate the resulting slurry into two streams. The first, liquid, stream contains fat, protein and water, and can be used in the manufacture of meat stocks and broths, as discussed later in this chapter (Section 4.4.3). A second, semi-solid, stream is usually dehydrated, after which it is ground, flaked, milled or granulated to obtain dry functional collagen ingredients (Jobling, 1994).

Uses and applications

One commercial pork collagen product (MyoGel Plus, Proliant Inc., Ankeny, IA; 85% protein, 12% fat), obtained by 'low temperature processing of fresh pork trimmings' in a process that involves extrusion and dehydration, followed by drying and milling into a granular form (Prabhu, 2002; Prabhu and Doerscher, 2000), has been reported to be capable of binding up to four times its weight in water. In one study, it was observed that addition of this ingredient to 22–23% fat frankfurters (at levels from 0 to 3.5% in 0.5% increments) and 3% fat restructured ham (at levels of 0, 1.0, 2.0, and 3.0%) helped control package purge over 8 weeks of refrigerated storage and slightly increased cook yields in frankfurters (by approx. 1% at up to 1.0%

addition, beyond which no significant increases were observed) but not in ham (Prabhu *et al*., 2004). In a separate study, this same product, at a usage level of 3% in boneless cured ham made with up to 100% PSE (pale, soft, exudative) meat, was observed to reduce expressible moisture, but not cooking loss. However, when only non-PSE meat was used, both cooking loss and expressible moisture decreased with the addition of pork collagen (Schilling *et al*., 2003).

In an effort to understand how concentrated, dehydrated pork collagen (MyoGel Plus, Proliant Inc.) interacts with myofibrillar proteins, one study assessed its effects on the thermal and viscoelastic properties of purified porcine myofibrillar protein gels (Doerscher *et al*., 2003). Replacement of 20% or more of the myofibrillar protein with pork collagen was reported to decrease the rate of gel formation, leading the researchers to suggest that pork collagen may interfere with the formation of the myofibrillar protein heat-set gel matrix. Usage levels of pork collagen were 0, 10, 20, 30 40, and 50%, with 10% being optimal in terms of water-holding capacity, gel firmness, and rate of gel formation. Specific protein-protein interactions between pork collagen and myofibrillar proteins, tested by differential scanning calorimetry (DSC) and oscillatory rheology, were not detected.

Low-temperature processing of poultry (chicken or turkey) skin has also been utilized commercially to obtain similar functional collagen proteins (>70% protein, <28% fat); it has been claimed that, due to their gelling and water-binding properties, these can increase cook yields and decrease formulation costs in various poultry products (Prabhu, 2002, 2003).

Certain extraction and/or treatment conditions can further modify the functional properties of collagen (Tarté, 2009). Precooking of high-collagen raw materials has been reported to increase their functionality in processed meat systems (Sadowska *et al*., 1980; Whiting, 1989), primarily due to the fact that it solubilizes early during the process, as opposed to native collagen, which generally melts and becomes gelatin too late in the process (i.e., between 75 and 80°C) and is, therefore, unable to become an integral part of the batter's gel structure. In one study (Osburn *et al*., 1997) pork skin connective tissue, obtained by cutting pork skin into strips, followed by freezing, grinding, refreezing and flaking, was heated in water at 50, 60, 70, or 80°C for 30 min. Under these conditions, gels produced by heating to at least 70°C had the highest water-binding ability. Addition of these 70°C gels in reduced-fat (2.0, 3.5, 4.3, 6.8, and 12.0% fat) bologna yielded varying results in terms of texture (hardness, juiciness) and processing yields.

Another application of collagen is in the manufacture of so-called 'casingless,' or co-extruded, sausages. This technology, the origins of which can be traced back to at least the 1960s (Hansen *et al*., 1962), consists of extruding an acid-swollen collagen batter around a continuous rope of sausage batter. After extrusion, the sausage rope is passed through a concentrated salt solution in order for osmotic dehydration of the collagen coating to take place, which allows the collagen to unswell, reassume a fibrous structure

and provide a strong 'casing.' The sausage rope is then cringed, dried, treated with liquid smoke, and cooked as necessary (Frye *et al*., 1996). Liquid smoke is important not just from a color and flavor standpoint, but also because it provides aldehydes that help form permanent collagen cross-links.

Regulatory aspects

In the US, beef and pork collagen are approved for use in standardized and non-standardized processed meat and poultry products in which binders are permitted, at a maximum level of 3.5% (USDA-FSIS, 2010).

In the EU, collagen is defined as 'the protein-based product derived from animal bones, hides, skins and tendons manufactured in accordance with the relevant requirements of [Regulation (EC) No. 853-2004]' (European Parliament and Council, 2004). It is not classified as a food additive and is therefore not subject to food additive legislation.

4.4.2 Gelatin and gelatin hydrolysates

Obtainment and manufacture

Gelatin is the product of the denaturation and partial hydrolysis of native, insoluble collagen. It is a soluble amorphous mixture composed mostly of three types of free chains: α monomers (mol wt 100 kDa), β dimers (mol wt 200 kDa), and γ trimers (mol wt 300 kDa) (Kijowski, 2001). During the gelatin manufacturing process, the collagen molecule's hydrogen bonds are disrupted and its intramolecular (aldol condensation and Schiff base), intermolecular and main-chain peptide bonds are hydrolyzed, causing its triple helix to unravel (Eyre, 1987) and leading to the disassembly of the collagen fibrils. The result is the viscous, colloidal solution known as gelatin.

Commercially, gelatin is obtained primarily from raw materials rich in type I collagen, primarily pork skin and bones, beef hides and bones and calf skin, through a controlled stepwise process that involves the chemical hydrolysis of collagen, followed by thermal denaturation. There are two types of processes for converting collagen to gelatin. In the acid process, type A gelatins are obtained by the mild acid pretreatment of physiologically young forms of collagen (e.g., pig skins, fish skin, certain types of bone), which have high proportions of acid and heat labile cross-links (Bailey and Light, 1989; Eyre, 1987; Stainsby, 1987). Type B gelatins are obtained by the more severe alkali process, in which more highly cross-linked collagen sources – typically those from more mature animals, such as cattle hides and bone – are pretreated with caustic soda or lime prior to extraction (Pearson and Gillett, 1999; Stainsby, 1987). The isoelectric point of type A gelatins is generally in the pH 6–9 range while that of type B gelatins is approximately pH 4.8–5.2. As a result, in most food systems type A gelatins carry a net positive charge, whereas type B gelatins are positively charged in acidic systems and negatively charged in near-neutral systems (Stainsby, 1987). Following the extraction step, gelatin is clarified

(by filtration), concentrated (by vacuum evaporation or membrane ultrafiltration), dried, ground, blended and, sometimes, sterilized (Linden and Lorient, 1999; Stainsby, 1987). Commercial gelatin extracts contain not only α, β, and γ chains, but also other larger (up to 106 kDa) and intermediate size molecules. The spectrum of molecular species and, as a consequence, the functional properties of the gelatin extract, are influenced by changes in the hydrolysis and extraction procedures as well as by the nature of the starting raw materials, with type A gelatins generally having a lower mean molecular weight than type B gelatins (Cole and Roberts, 1996; Stainsby, 1987).

The properties of gelatin can be modified further by controlled enzymatic hydrolysis of gelatin solutions to reduce the protein's molecular weight to a desired range. The resulting gelatin hydrolysates possess properties similar to gelatin with the exception that, due to their lower mean molecular weight, they disperse more easily in cold water and do not gel at regular processing temperatures.

Functional properties

Although gelatin is deficient in methionine and completely devoid of tryptophan (Bailey and Light, 1989), both essential amino acids, it possesses excellent functional properties, such as gelling, melting (melting temperature <35°C), stabilization, film-forming, texturizing, and water-holding properties, which make it a very useful and desirable food ingredient for many applications. Its gels, like those of collagen, are thermoreversible. Typically, the gel strength and viscosity of gelatin solutions decrease with decreasing mean molecular weight (Cole, 2000), which suggests that gelatin extracts must be chosen very carefully on the basis of the functional properties desired for a particular application. Other important factors to consider when choosing a gelatin are flavor, aroma, color and particle size.

The characterization, grading, and commercialization of dried gelatins is done on the basis of their gel strength expressed in Bloom units. A Bloom unit is defined as the force in grams required to press a 12.5 mm diameter flat-faced, sharp-edged, cylindrical probe 4 mm into 112.5 g of a 6⅔% (w/v) gelatin gel that has been aged 16–18 h at 10°C (Gelatin Manufacturers Institute of America, 2006). Gelatin Bloom values typically range from around 100 Bloom for very weak gels to around 250 Bloom for firm gels (Rosenthal, 1999).

Uses and applications

In foods, the more traditional uses of gelatin have been in such products as jams and jellies (for its reversible gelling properties), confectionery (as a binder), marshmallows (as a foam stabilizer), yogurt (as a stabilizer), dried soups (to provide mouthfeel), fruit juices (for its clarifying properties) and processed meat products such as aspics, canned hams and canned sausages (to add flavor and improve appearance), to name a few (Bailey and Light,

1989; Cole, 2000; Stainsby, 1987). It is also used in pharmaceutical capsules, taking advantage of its film-forming properties (Cole, 2000). More recent applications have seen its use in nutraceuticals, high-protein nutrition bars (in conjunction with dairy and vegetable proteins), and powdered and ready-to-drink beverages (Taffin and Pluvinet, 2006). Gelatin and gelatin hydrolysates have also recently been proposed as external coatings to protect meat against color loss, aroma deterioration and purge losses (Antoniewski *et al*., 2007; Krochta and De Mulder-Johnson, 1997; Villegas *et al*., 1999), a protective effect that has been attributed to the action of gelatin as a moisture and oxygen barrier.

Regulatory aspects

In the US, gelatin has Generally Recognized as Safe (GRAS) status. In meat and poultry products it is permitted as a binder and extender in various products at levels sufficient for the intended purpose (CFR, 2010k). Its permitted uses include non-specific products, jellied products (e.g., souse, jellied beef loaf, head cheese), paté-type products (as a covering; product name qualifier required in red meat paté products), canned whole hams (requires product name qualifier), and products where 'gelatin' is part of the product name. It may also be used to bind two pieces of meat together but is not permitted in sausage, luncheon meat, and meat loaves (USDA-FSIS, 2005). Hydrolyzed gelatin is also recognized as a binder rather than a flavoring and is permitted in frankfurters and similar products, as well as poultry frankfurters, at usage levels of 2% or less (USDA-FSIS, 2005).

In the EU, gelatin is classified as a food, not a food additive (European Parliament and Council, 2006), and is therefore not subject to food additive legislation. It is not considered meat and must, therefore, be declared separately.

4.4.3 Stocks and broths

Obtainment and manufacture

Commercial meat stocks and broths are derived from the liquid stream of the low-temperature rendering of soft materials such as meat lean and fatty trimmings, pig skins, and poultry skins (as previously described in the section on collagen) or the high-temperature rendering of hard materials such as edible bones (Campbell and Kenney, 1994). During its manufacturing process the fat is separated from the protein-containing liquid stream, which is then concentrated and spray-dried (Jobling, 1994; Prabhu and Hull, 2001) to a protein content >94%. Meat stock proteins are collagenous in nature.

Functional properties

Meat stocks do not possess strong rheological functional properties, due to the fact that their proteins are extensively hydrolyzed. However, their high

content of low molecular weight volatile and nonvolatile compounds does make them highly functional as flavoring agents and flavor enhancers. Many of these compounds can in turn act as precursors of other volatile compounds that develop during cooking of the food product (Cambero *et al*., 2000; Hornstein and Wasserman, 1987; Melton, 1999; Mottram, 1998; Snitkjær *et al*., 2010).

Uses and applications
Meat stocks can be used as bases for the manufacturing of meat and reaction flavors, as well as flavoring agents and flavor enhancers in soups, sauces, processed meat products and other savory foods. They can be used to increase protein content in sausage products and in some instances can act as emulsifiers, binders and stabilizers, although that is not usually their primary purpose in processed meat product systems.

Regulatory aspects
In the US, current USDA regulations [9 CFR 317.8(b)(7)(ii)] mandate that 'ingredients of livestock and poultry origin must be designated by names that include the species and livestock and poultry tissues from which the ingredients are derived' (CFR, 2010b). Therefore, ingredients such as dried stocks, dried broths, and meat extracts must be designated as 'dried (species) stock,' 'dried (species) broth,' and '(species) extract' (e.g., 'dried chicken stock,' 'dried beef broth,' 'pork extract,' etc.) (USDA-FSIS, 2006a).

4.5 Hydrolysates and flavors

4.5.1 Obtainment and manufacture

Meat protein hydrolysates encompass a broad family of products that can be obtained from meat by-products such as bone residues, mechanically separated meat (MSM), bone residues from mechanical separation, trimmings (Fonkwe and Singh, 1996; Webster *et al*., 1982), blood plasma (Wanasundara *et al*., 2002), and red blood cells (Shahidi *et al*., 1984; Synowiecki *et al*., 1996), as well as from skeletal muscle and connective tissue. The possibilities and opportunities in this area are potentially great, given the different kinds of potential raw material sources available which can, in turn, be hydrolyzed to varying extents to yield different types of functional products. Therefore, although some hydrolysates have already been discussed (i.e., gelatin and gelatin hydrolysates), this topic is worth expanding and discussing further.

Hydrolysis can be achieved by treatment with enzymes, acids, or alkali (Lahl and Braun, 1994), but for many applications the enzymatic process is preferred due to its faster reaction rates, mild process conditions, and high specificity (Hamada, 1992), and because it allows for more precise control of the degree of hydrolysis (DH) and, as a result, of the peptide and amino

acid profile of the resulting hydrolysates (Lahl and Braun, 1994). Degree of hydrolysis, usually expressed as the ratio of amino nitrogen to total nitrogen (AN/TN), or percent of peptide bonds cleaved, is a measure of the extent of hydrolytic degradation of proteins (Mahmoud, 1994). It is both a practical and effective way of monitoring and controlling the hydrolysis process, and a good indicator of a hydrolysate's functional properties (e.g., solubility, gelation, water holding, emulsification, flavor, etc.).

Meat hydrolysates can be classified as either primary (partially hydrolyzed) or secondary (extensively hydrolyzed). Primary hydrolysates result from hydrolysis by one or more endopeptidases of animal (e.g., pepsin, trypsin, chymotrypsin), vegetable (e.g., papain, bromelain), bacterial (e.g., subtilisin from *Bacillus subtilis*, *Bacillus amyloliquefaciens*, or *Bacillus licheniformis*), or fungal (endoprotease from *Aspergillus oryzae*) origin (Piette, 1999; Pinto e Silva *et al*., 1999). A secondary hydrolysis may be necessary in order to break down bitter peptides that may form as a result of partial hydrolysis (Pedersen, 1994). In these cases, exopeptidases are utilized, which can also be of animal, bacterial (e.g., *Bacillus* spp.), or fungal (e.g., aminopeptidases from *Aspergillus* spp.) origin, and are generally more effective after endopeptidases have reduced the average peptide size.

4.5.2 Functional properties

In general, properties such as emulsion stability, viscosity, and gel-forming ability decrease with increasing DH, due to the smaller molecular weight and to the increased net charge that results from hydrolysis (Mahmoud, 1994). Conversely, as DH increases, the hydrolysates' flavor contribution increases, primarily owing to the presence of low-molecular weight flavor components (e.g., amines, amino acids, and small peptides) and flavor precursors (e.g., organic acids and nucleotides). Therefore, extensive hydrolysis will result in products of such low rheological functional quality that they become strictly limited to use as flavors and flavor enhancers, and for protein supplementation (Synowiecki *et al*., 1996). In many cases this is desirable. Meat flavor notes can also be obtained from meat stocks and broths, as discussed previously, either by enzymatic hydrolysis or by reacting them with certain Maillard reactants (e.g., reducing sugars). Other factors that will affect the end-product obtained include the specificity of the hydrolytic enzymes used, the physicochemical nature of the intact parent protein, and processing conditions (Mahmoud, 1994).

4.5.3 Uses and applications

Knowledge of the extent and type of the hydrolytic reactions involved in the manufacture of meat protein hydrolysates allows the process to be manipulated and controlled to yield products with specific functional

attributes, as described previously. The choice of meat protein hydrolysate is thus dictated by the specific functional properties desired for each particular application and may also be limited by commercial availability. Commercially, meat protein hydrolysates can be concentrated and used as added ingredients in liquid or powder form (Piette, 1999).

4.5.4 Regulatory aspects

In the US, the Food and Drug Administration (FDA) requires that '[t]he common or usual name of a protein hydrolysate shall be specific to the ingredient and shall include the identity of the food source from which the protein was derived' [21 CFR 102.22] (CFR, 2010j). For meat protein hydrolysates this requirement is also mandated by 9 CFR 317.8(b)(7)(ii), which states that 'ingredients of livestock and poultry origin must be designated by names that include the species and livestock and poultry tissues from which the ingredients are derived' (CFR, 2010b). Consistent with these regulations, the USDA requires that 'hydrolyzed protein of slaughtered animal species and tissue of origin, other than gelatin, must be indicated, e.g. 'hydrolyzed beef plasma,' 'hydrolyzed pork stock,' and 'hydrolyzed pork skin' (USDA-FSIS, 1995a). The degree of hydrolysis of the material also has labeling implications. Proteins with AN/TN ratios greater than 0.62 are considered by the FDA to be 'highly' hydrolyzed and must be declared as 'hydrolyzed (source protein).' Proteins with AN/TN < 0.62 are not considered highly hydrolyzed and may therefore be declared as 'partially,' 'mildly,' or 'lightly' hydrolyzed (e.g., 'partially hydrolyzed [source protein]') (USDA-FSIS, 1995a).

Regarding usage limits, partially hydrolyzed proteins are permitted in the US in various meat and poultry products at a maximum level of 3.5% (USDA-FSIS, 2010).

In the EU, protein hydrolysates and their salts are not classified as food additives (European Parliament and Council, 2006) and are, therefore, not subject to food additive legislation. They are not considered meat and must, therefore, be declared separately.

4.6 Blood protein ingredients

4.6.1 Obtainment and manufacture

Blood makes up approximately 7% of the bodily weight of mammals (Judge *et al*., 1989); however, its recovery during slaughter of cattle and hogs is about 3–4% of live weight (Liu and Ockerman, 2001). Historically, whole blood has been used as an ingredient in various meat products, such as blood sausage and blood pudding, among others. Bovine or porcine blood has also been used as a raw material for the production of a wide range of functional ingredients.

Table 4.7 Composition of blood and of its major fractions

Blood fraction	Protein (%)	Moisture (%)
Whole blood	17–18	75–82
Plasma	6–8	90–92
Cells	34–38	60–62
Dried plasma	70–95	5–10

In order to produce functional blood ingredients, blood is typically first separated into two fractions: plasma (60–80%) and cells (20–40%; mostly red cells, with smaller amounts of white cells and platelets) (Liu and Ockerman, 2001). Separation of the two fractions is generally accomplished by continuous high-speed centrifugation or separation, and for this process to be successful blood collection must be done promptly after slaughter, generally within 20 min (Halliday, 1973), and being careful to minimize hemolysis of the red cells, since release of hemoglobin could make it impossible to separate the plasma (Knipe, 1988). In addition, anticoagulants such as citric acid or sodium citrate are usually added at this stage. After separation, the plasma fraction is usually either frozen, or concentrated and spray-dried.

Table 4.7 shows the composition of whole blood and of its constituent fractions. Blood proteins are deficient in the essential amino acids methionine and isoleucine (Penteado *et al.*, 1979; Satterlee, 1975; Tybor *et al.*, 1975; Wismer-Pedersen, 1979) and their levels in blood can vary with age and animal species (Gorbatov, 1988). Whole blood and red blood cells (which are discussed later) have traditionally found only limited application as food ingredients (Piot *et al.*, 1986; Wismer-Pedersen, 1979), primarily due to their dark color and unpalatability. The plasma fraction, on the other hand, due to its more desirable color and functional properties, has attracted more interest over the years (Knipe, 1988).

4.6.2 Blood plasma proteins

Blood plasma contains well over 100 different proteins, the major ones being the serum proteins albumin, α-globulins, β-globulins, and γ-globulins; and fibrinogen (Table 4.8). Of these, serum albumin is the most abundant and the most important from a commercial point of view. In its dehydrated form blood plasma protein is an off-white powder with very little pigmentation.

Functional properties, uses and applications

Research on the use of blood plasma protein (BPP) as a functional ingredient (primarily for water-holding and binding) in meats dates to at least the

Table 4.8 Basic properties of the major plasma proteins.[1] Adapted from Gorbatov (1988) and Howell (1992)

Protein	% of plasma protein	pI	Molecular weight (kDa)
Serum albumin	56	4.8–4.9	69
α_1-Globulins	5.3	2.7–4.4	44–435
α_2-Globulins	8.4	3.6–5.6	41–20 000
β-Globulins	11.5	3.6–5.9	80–3200
γ-Globulins	15	5.8–7.3	100–160
Fibrinogen	0.6	–	340

[1] Protein levels vary with animal species and age.

late 1970s (Dill and Landmann, 1988). Various studies have attributed its functional properties primarily to its albumin, globulin, and fibrinogen content (Chen and Lin, 2002; Dàvila *et al*., 2007; Foegeding *et al*., 1986a). In addition, it has been suggested that BPP likely contains protease inhibitors, given the finding that as little as 0.5% in surimi has been shown to reduce degradation of myosin (Lou *et al*., 2000; Wang *et al*., 2000).

Blood plasma protein possesses three primary functional attributes which make them particularly useful ingredients in food products: gelation, emulsification, and solubility:

- *Gelation*. Suspensions of 4–5% BPP form strong, irreversible gels (Hermansson, 1978) when heated to a minimum of 70°C. This gelation behavior is dependent on several factors, primarily temperature, pH, heating time, and protein concentration. While BPP begins to gel at approximately 70°C, firmer gels can be formed by increasing the temperature to 90–92°C (Harper *et al*., 1978; Hermansson and Lucisano, 1982), with even stronger gels being reported at temperatures as high as 95°C (Foegeding *et al*., 1986a). This behavior makes BPP a useful ingredient in products that are subjected to very high processing temperatures, such as canned items and certain restructured meat products (Terrell *et al*., 1982; Xiong, 2004). The firmness of BPP gels may also be increased by increasing cooking time and protein concentration (Harper *et al*., 1978; Hermansson, 1978, 1982), as well as by the addition of sodium chloride (Hermansson, 1978, 1982), with a level of at least 1.5% being necessary to attain an acceptably firm gel (Harper *et al*., 1978; Knipe, 1988). Various properties of porcine blood plasma have been observed to be pH-dependent. In one study (Dàvila *et al*., 2007), gelation temperature, gel hardness, elasticity, cohesiveness and water-holding capacity all increased as pH increased from 4.5 to 7.5. In another study, water-binding in mixed bovine/porcine gels was also reported to be higher at pH 9.0 than at pH 7.0 (Hermansson and Lucisano, 1982).

- *Emulsification*. Although most comminuted meat products are never subjected to temperatures above BPP's gelation temperature of 70–75°C, the excellent emulsifying properties of BPP (Tornberg and Jönsson, 1981) still make it ideal for use in these products (Ockerman and Hansen, 2000; Prabhu, 2002; Terrell *et al.*, 1979). BPP has been reported to improve the emulsion stability, texture, flavor, juiciness, and peelability of comminuted meat products (Prabhu, 2002).
- *Solubility*. Plasma protein exhibits high solubility over the pH range 5.0–8.0 (Hermansson, 1978; King *et al.*, 1989), making it ideal for use in many food products where solubility is important. Since most meat products fall in this pH range, it is an ideal binder for use in many meat processing situations, especially where solubility is critical, such as brines and marinades. In addition, BPP has also been successfully used as a binding agent in sausage (Caldironi and Ockerman, 1982) and ground beef patties (Suter *et al.*, 1976).

Plasma protein fractions

Plasma can be further fractionated into its major constituents, namely albumin, globulins, and fibrinogen. Precipitation and removal of fibrinogen leaves behind serum, which can then be further fractionated into albumin and globulins.

Much research has been done on the functional properties of these individual fractions. The gelation and other functional properties of albumin and fibrinogen have been investigated (Foegeding *et al.*, 1986a, 1986b), as well as the potential utilization of specific plasma fractions as food ingredients (Chen and Lin, 2002; Dàvila *et al.*, 2007; Penteado *et al.*, 1979). A synergistic effect between the different fractions of plasma has been suggested by various studies (Howell and Lawrie, 1984). In one study (Penteado *et al.*, 1979) the oil emulsification capacity of 1% solutions of bovine blood plasma proteins was observed to be plasma > albumin > globulins, whereas in another (Ramos-Clamont *et al.*, 2003) oil-in-water emulsions were more stable when made with serum than with albumin alone (for both beef and pork blood-derived fractions), up to 14 days of storage at 25°C. A similar synergistic effect has also been observed in relation to the gelation behavior of plasma and its fractions. In an aforementioned recent study of the effects of pH on the heat-induced gelation of porcine albumin, serum and plasma (Dàvila *et al.*, 2007), 5% gels made from each of these three fractions became progressively weaker as pH decreased from 7.5 to 4.5, with albumin gels being much weaker than serum and plasma gels, despite the fact that albumin is plasma's most abundant component. Serum gels were weaker than plasma gels at pH 7.5. As pH was decreased from 6.0 to 4.5 both gels became weaker, but serum gels were now stronger than plasma gels, an effect that was attributed to the presence of fibrinogen in plasma, and which suggests that fibrinogen may have detrimental effects on the functional performance of these proteins in low-pH food systems.

Regulatory aspects

The use of blood plasma in meat products is permitted in the US, where current regulations require that it be identified on the product label by its common or usual name and that its species of origin be identified (e.g., 'dried [species] plasma') (Post *et al.*, 2007; USDA-FSIS, 2006a).

In the EU, blood plasma is not considered a food additive (European Parliament and Council, 2006), and is therefore not subject to food additive legislation. It is not, however, considered meat and must, therefore, be declared separately.

4.6.3 Plasma transglutaminase

Functional properties

Transglutaminases (TGases; EC 2.3.2.13) are thiol enzymes that catalyze acyl transfer reactions in which γ-carboxamide groups of peptide-bound glutaminyl residues act as acyl donors and primary amines act as acyl acceptors. When the acyl acceptors are the ε-amino groups of lysine residues, inter- and intra-molecular ε-(γ-glutamyl)lysyl covalent cross-links are formed (Fig. 4.2) (de Jong and Koppelman, 2002; Folk, 1980; Griffin *et al.*, 2002; Motoki and Seguro, 1998). TGases have been found in plants, bacteria, fish, mammals, birds, and amphibians; however, to date only those obtained from bacteria (Zhu *et al.*, 1995) and mammalian plasma (blood clotting factor XIIIa) can be produced in quantities large enough, and demonstrate cross-linking activity of native proteins (Table 4.9) adequate enough, to make them commercially viable.

Uses and applications

TGases effectively cross-link casein, whey proteins, soy proteins, wheat proteins, myosin, actomyosin, gelatin, and collagen (Piette, 1999), although activity and substrate specificity are dependent on the origin of the enzyme and the state of the substrate protein chain (Table 4.8), as well as on reaction conditions such as temperature and pH (Kurth and Rogers, 1984). Plasma and erythrocyte TGases require Ca^{2+} as a cofactor, whereas bacterial TGase is calcium-independent (de Jong and Koppelman, 2002).

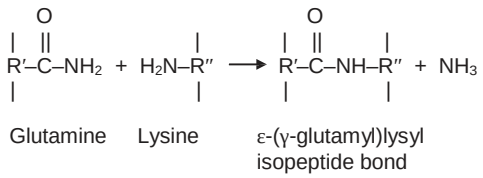


Fig. 4.2 Transglutaminase-catalyzed cross-linking reaction between peptide-bound glutamine and lysine.

Table 4.9 Food protein substrate specificity of transglutaminases of different origin[1]

	Degree of cross-linking[2,3]					
	Pig erythrocyte TGase		Bovine plasma TGase		Bacterial TGase	
	−DTT	+DTT	−DTT	+DTT	−DTT	+DTT
α-Lactalbumin	−	±	−	±	+	++
β-Lactoglobulin	−	−	−	±	−	++
Bovine serum albumin	−	+	−	+	−	++
Casein	−	++	++	++	++	++
Hemoglobin	−	−	±	±	±	±
Myosin	−	−	++	++	++	++
Glycinin	−	++	−	−	++	++

From de Jong *et al.* (2001).
[1] Experimental conditions: 37°C; pH 7.5.
[2] Symbols: (−) no cross-linking; (±) slow cross-linking; (+) moderate cross-linking; (++) fast cross-linking.
[3] DTT: Dithiothreitol; promotes unfolding of the protein chain by reducing disulfide bridges.

Plasma TGase can be utilized to bind pieces of raw meat, thus enabling processors to increase the economic value of lower-value cuts and trimmings by converting them into higher-value restructured products of uniform portion size, shape, and texture (Flores *et al.*, 2007; Nielsen *et al.*, 1995; Paardekooper and Wijngaards, 1986). It has also been used to improve the texture of sausages, alone (via cross-linking of meat proteins) (Muguruma *et al.*, 1999) or in combination with other non-meat proteins, such as casein or soy protein (Kurth and Rogers, 1984). In addition, plasma TGase may offer a technically viable way to reduce sodium in meat products (Tseng *et al.*, 2000) and to replace food additives such as phosphates (Muguruma *et al.*, 2003).

Currently the only commercially available system that takes advantage of plasma TGase is Fibrimex® (Sonac BV, Loenen, Netherlands), which combines the glycoprotein fibrinogen with the enzyme thrombin (Paardekooper and Wijngaards, 1986). Fibrimex® is available in frozen liquid and powder forms (Sonac BV, 2010). Its liquid version consists of the following two components: (i) a preparation of bovine or porcine blood plasma (which contains zymogen Factor XIII, or fibrin-stabilizing factor) to which partially-purified fibrinogen has been added, and (ii) a calcium chloride ($CaCl_2$)-containing solution of the enzyme thrombin (coagulation factor II; EC 3.4.21.5), also extracted from bovine or porcine blood plasma. Immediately prior to addition to meat, these two components are mixed in a specified ratio of 20 : 1, respectively. The dry powder can be added directly or it can be pre-mixed with water to ensure better dispersion. After incorporation of the Fibrimex® components, the meat mixture must be held at

0–4°C for at least 6 h for optimal binding (Sonac BV, 2010). During this holding time thrombin catalyzes the breakdown of fibrinogen to fibrin monomers, which polymerize and form a gel, and the proteolytic activation of Factor XIII into its active transglutaminase form, factor XIIIa, which, due to the presence of Ca^{2+}, forms covalent cross-links between individual fibrin molecules, as well as between fibrin and fibronectin, fibrin and collagen (Piette, 1999), fibrin and actin, myosin and actin, and myosin and fibronectin (Kahn and Cohen, 1981). Of these, fibrin-fibrin, fibrin-fibronectin, and fibrin-collagen cross-links appear to be the most important in meat applications (Piette, 1999). Strong binding between meat pieces results from the combined effect of these cross-links and of fibrin.

Regulatory aspects

In the US 'beef fibrin,' defined as 'a component mixture of beef fibrinogen and beef thrombin plasma protein used to bind pieces of meat or poultry together,' is permitted at up to 10%. In terms of labeling, the words 'Formed with Beef Fibrinogen and Thrombin' must appear either in the product name (at usage levels of 7–10%) or in the product name qualifier (at usage levels of less than 7%) (USDA-FSIS, 2005).

In the EU, despite having been previously declared safe by the European Food Safety Authority (European Food Safety Authority, 2005), the European Parliament recently rejected a draft Commission Directive that would have added bovine and/or porcine thrombin to the list of food additives (European Parliament, 2010) on grounds that 'the use of thrombin with fibrinogen as a food additive could mislead the consumer as to the state of the final food' and that 'the process of binding together many separate pieces of meat significantly increases the surface area that may be infected by pathogenic bacteria (such as clostridium and salmonella) which, in such a process, can survive and be reproduced without oxygen,' among other justifications given. It presently remains to be seen whether individual Member States will choose to approve its use as a 'processing aid.'

4.6.4 Hemoglobin and red blood cells

Functional properties

Hemoglobin makes up approximately 70% of total blood protein. Since it is found in red blood cells (erythrocytes), when the plasma and cell fractions of blood are separated, most of it remains with the cellular, or corpuscular, fraction.

Uses and applications

As previously mentioned, the use of hemoglobin and hemoglobin-rich materials as ingredients in food products, including meats, has been limited, primarily because of the dark color and off-flavors they impart. In order to overcome this limitation, attempts have been made to decolorize

hemoglobin. This can be achieved by treating it with hydrogen peroxide (H_2O_2) (Oord and Wesdorp, 1979), acid-acetone solution (Antonini and Brunori, 1971), carboxymethylcellulose chromatography (Sato *et al*., 1981), enzymatic hydrolysis (Stachowicz *et al*., 1977), and aluminum oxide (Piot *et al*., 1986). Another attempt at overcoming the disadvantages of hemoglobin involves removal of the heme group (Tybor *et al*., 1973, 1975). Although the resulting globin has good water-holding capacity, its effectiveness is limited by the fact that it does not form a gel when heated.

The color imparted by hemoglobin and red blood cells can be advantageous when color enhancement is desirable. Stabilized hemoglobin products, in both liquid and powder form (Sonac BV, 2007), as well as spray-dried red blood cells, are or have been commercially available for this purpose, mainly in countries other than the US. Another interesting development involves treating red blood cells – or a hemin intermediate isolated from them – with a nitrosating agent (typically nitric oxide) in the presence of a reductant to produce a mononitrosyl derivative of reduced hemin referred to as Cooked Cured-Meat Pigment (CCMP), which has been proposed as a coloring agent in composite nitrite-free processed meat systems (Pegg and Shahidi, 2000). As of this writing, however, this product has not been commercialized.

Regulatory aspects

In the US, blood is permitted in blood sausage, blood pudding, blood soup, and in beef patties, as long as a qualified product name is used, e.g., 'Beef and Blood Patties' or 'Beef Patties with Blood'. A coating of beef blood is permitted on cured products (e.g., ham, hamette, etc.) if the product name is prominently qualified to reflect the coating (USDA-FSIS, 2005). In all products in which blood is permitted, the term 'blood,' and the species name shall be declared in the ingredient statement, e.g., 'beef blood' or 'sheep blood' [9 CFR 317.8(b)(31)] (CFR, 2010b).

4.7 Future trends

In addition to the specific functional properties discussed in this chapter, meat protein ingredients provide other more general, but important, advantages when used to formulate food products. Two of these are worth highlighting. First, they are considered non-allergenic, which makes them good potential options for the replacement of commonly-used allergenic proteins, such as dairy and soy. Second, because they are generally 'minimally-processed,' they do not possess chemical-sounding names that may alienate some consumers. This makes them more consumer-friendly and label-compatible than many other ingredients, which could be advantageous to many food processors in their quest for 'simpler' and 'cleaner' ingredient declarations.

As is always the case when deciding on the best ingredients to use for a particular application, the final decision always comes down to a thorough cost vs. benefit analysis. Therefore, careful design of the food product's desired quality attributes (texture, color, flavor, shelf-stability, label, etc.) and cost structure will, ultimately, determine which ingredients are most suitable to use. In order to arrive at this end, competing ingredients must be carefully tested and selected, based on their functional attributes and price. To this end, the reader is encouraged to consult many of the excellent references given in this chapter for further advice.

4.8 Acknowledgment

Portions of the material presented in this chapter have been previously published (Tarté R, 2009, Meat-derived protein ingredients, chapter 7 in Tarté R, *Ingredients in Meat Products: Properties, Functionality and Applications*, 145–171, © Springer Science+Business Media, LLC 2009) and have been reprinted with kind permission of Springer Science+Business Media.

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6

Seafood proteins

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Abstract: Seafood is a source of high quality proteins valued for their functional properties and nutritional value. This chapter first discusses basic chemistry and nutritional value of seafood proteins, followed by description of their practical functional properties and factors that affect them. Due to over-exploitation of marine resources and growth of human population, it is desirable to devise novel processing strategies to efficiently recover proteins from seafood and seafood processing by-products. This chapter also sheds light on new techniques to address protein recovery from seafood. Finally, this chapter includes seafood protein applications and environmental consideration of sustainable seafood production.

Key words: seafood proteins, protein functional properties, seafood protein applications, protein recovery, seafood sustainability.

6.1 Introduction

Seafood is the only source of animal protein that is still provided in significant amounts to human diet through capture of wild species (Table 6.1). Seafood is an excellent source of high quality protein that contains sufficient amounts of essential amino acids (EAA) required in the human diet. Not only are all EAA provided in sufficient quantity, but also seafood protein is easily digestible and absorbable (Sánchez-Alonso *et al.*, 2007). Therefore, seafood protein has a high biological value (Venugopal, 2009; Tou *et al.*, 2007). However, the amino acid composition of seafood is influenced by intrinsic and extrinsic factors. The nutritional value and overall health benefits of fish protein is very important in consumer acceptability of fish products (Schwarz *et al.*, 1998). For this reason, dietary recommendations to the public from several authorities indicate that seafood should be an integral component of a healthy diet, particularly as it can replace other protein-rich food products that are high in saturated fat and dietary cholesterol (Venugopal, 2009).

Table 6.1 World fisheries and aquaculture production and utilization[1] (adapted from FAO, 2009)

	2002	2003	2004	2005	2006
	million tonnes				
Production					
Capture	93.2	90.5	94.6	94.2	92.0
Aquaculture	40.4	42.7	45.9	48.5	51.7
Total fisheries	134	133	141	143	144
Utilization					
Human consumption	101	103	105	107	110
Non-food uses	32.9	29.8	36.0	35.6	33.3
Population (billions)	6.3	6.4	6.4	6.5	6.6
Per capita fish food supply (kg)	16	16.3	16.2	16.4	16.7

[1] Note: Excluding aquatic plants.

This chapter reviews functional properties of seafood proteins and factors that affect them. In addition, seafood proteins have specific biological properties, which make them potential ingredients in the development of health-promoting food products. This chapter discusses the specific functional properties of seafood proteins and protein supplements derived from seafood. In addition, along with an increasing awareness that our marine resources are not endless, numerous efforts have been undertaken to better utilize fish processing by-products and underutilized low-value species. Furthermore, the growth of aquaculture industry necessitates development of technologies that recover muscle proteins. Therefore, developing new technologies for the full utilization of seafood is of critical importance to the future economic viability of the seafood industry (Gildberg, 2002). This is why a section of this chapter discusses recovery of fish muscle proteins from fish processing by-products and underutilized low-value aquatic species using novel isoelectric solubilization/precipitation.

6.2 Chemistry of seafood proteins

6.2.1 Muscle structure in seafood: striated vs smooth muscle

Muscle tissue is the edible part of fish used in processing to derive various food products. These muscles are in general similar to those from terrestrial animals in terms of structure, composition and function. Two muscle types are present in seafood, striated muscle characterized by transverse stripes, and smooth muscle that lacks them (Torres *et al.*, 2007). Striated muscle is subsequently divided into two groups: white and dark meat. White meat (ordinary muscle) exists in all parts of the seafood, but dark meat can be

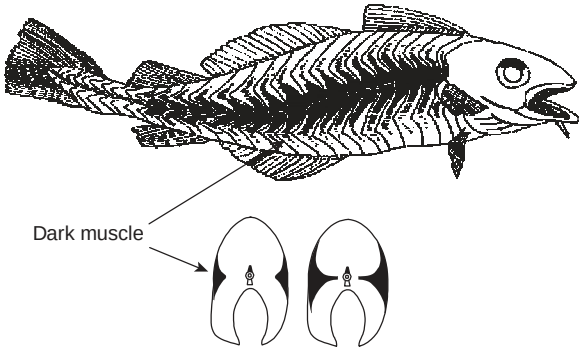


Fig. 6.1 A general diagram and a cross-section of fish illustrating distribution of dark and white muscle (adapted from http://www.emeraldinsight.com/fig/0170960108002.png).

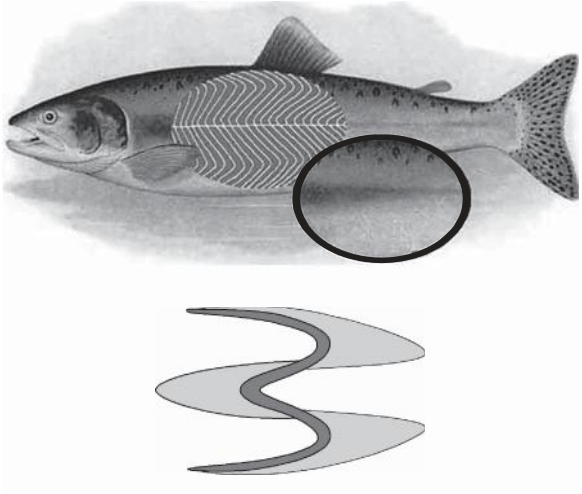


Fig. 6.2 A general diagram of fish muscle (top) and a single fish muscle block (myotome, bottom) illustrating myotome folding. Myotomes are separated by connective tissue (myosepta) (adapted from http://www.seaworld.org/infobooks/BonyFish/images/muscle.gif).

found underneath the skin as shown in Fig. 6.1. The quantity of dark meat varies from species to species. White meat consists of separate blocks called myotomes or myomers (Fig. 6.2). The blocks are held by a connective tissue called myocommata or myosepta.

Raw uncooked fish muscle is semi-transparent and it is composed of numerous fibers (Brown, 1986). Muscle fibers, smaller units of muscle structure are bound together by connective tissue (endomysium) and covered

with myocommata. Muscle fibers consist of many myofibrils which lie parallel to each other. The space between myofibrils is filled by sarcoplasmic proteins (Suzuki, 1981; Lanier *et al*., 2005).

6.2.2 Classification of seafood proteins

Proteins are the most important part of seafood muscle tissue and account for 15–25% of the total weight (wet weight basis). Fish proteins can be divided into three major groups according to their water solubility characteristics, myofibrillar proteins, sarcoplasmic proteins, and stroma proteins (Connell, 1980).

Myofibrillar proteins

Myofibrillar proteins are the proteins that form myofibrils. They are soluble in concentrated saline solutions (ionic strength above 0.6) as well as extremely low ionic strength, but are water insoluble in typical physiological ionic strength in the fish muscle (ionic strength approximately 0.05 for rainbow trout). Myofibrillar proteins are composed of myosin, actin, and regulatory proteins such as tropomyosin, troponin and actinin (Fig. 6.3). Myofibrillar proteins make up 66–77% of total proteins in fish muscle and provide several functional properties that are useful in food products. Generally, seafood myofibrillar proteins are less thermally stable than the proteins isolated from terrestrial animals. The pH and ionic strength affect thermal stability of seafood myofibrillar proteins, and hence, heat-induced denaturation. Myofibrillar proteins isolated from cold water species are typically less thermally stable than warm water species. This property translates into different requirements for handling and freezing of seafood from cold and warm waters. Protein gelation and rheological properties responsible for texture development, and therefore, consumer acceptability depends mainly on the quality of myofibrillar proteins, which is affected by seafood species, age, seasonality, freshness, and processing parameters such as protein concentration, pH, ionic strength and temperature (Suzuki, 1981).

Actin constitutes about 20% of the total amount of myofibrillar proteins in fish muscle. Actin is easily extracted. However, this characteristic presents a problem when pure myosin is to be isolated, because the extracted actin spontaneously forms actomyosin complex in the solution and hinders isolation of pure myosin. Therefore, actomyosin is the main form of salt-soluble fish muscle proteins. Tropomyosin and troponin regulate muscle contraction. The molecular weight of tropomyosin is 68 kDa and it has two subunit chains. Tropomyosin is the most heat stable muscle protein and is easily purified. Troponin is a necessary protein for tropomyosin to act as a relaxation factor during muscle contraction. Water solubility of myofibrillar protein varies depending on the temperature, pH, and ionic strength. Extreme pH and high temperature cause protein denaturation resulting in low solubility (Suzuki, 1981).

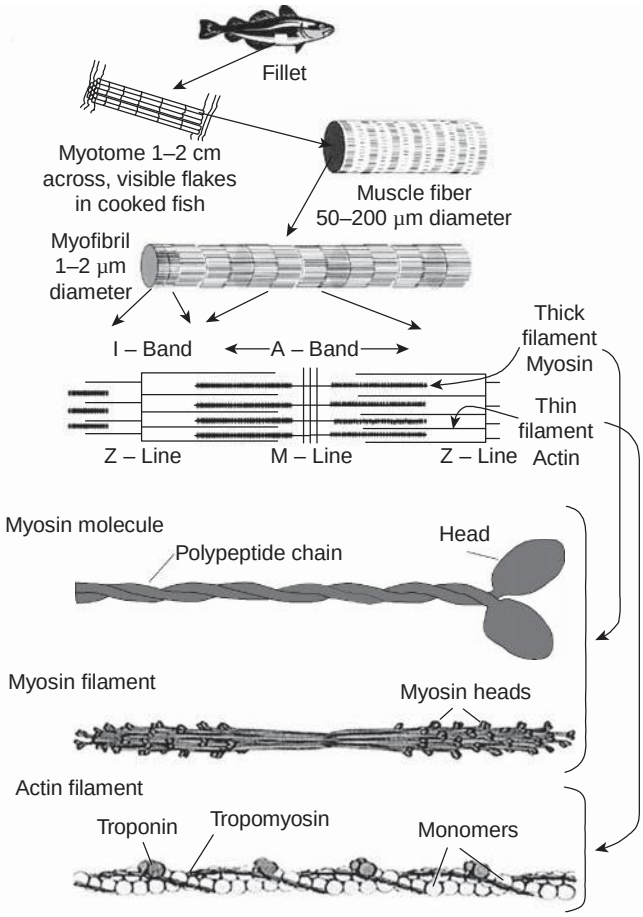


Fig. 6.3 Structure of muscle tissue (adapted from Bremner, 2002 and http://www.ucl.ac.uk/~sjjgsca/MuscleSarcomere.gif).

Sarcoplasmic proteins

Sarcoplasmic proteins contain several individual types of water-soluble proteins called myogen. Since sarcoplasmic proteins are completely water-soluble, they are isolated from fish muscle by simply pressing the fish muscle tissue or by extraction with low ionic strength saline solution. Pelagic fish such as sardine and mackerel have generally higher content of sarcoplasmic proteins compared to demersal fish like plaice and snapper. These proteins may interfere with myosin cross-linking during gel matrix formation because they do not gel and have poor water-holding capacity (Sikorski *et al*., 1994). To the contrary, more recent data shows that sarcoplasmic proteins may actually enhance thermal gelation of myofibrillar proteins. There are

probably several factors that govern heat-induced gelation of myofibrillar proteins in the presence of sarcoplasmic proteins. This area is currently being actively investigated by several laboratories and likely this phenomenon will soon be more definitely elucidated. Sarcoplasmic proteins comprise myoglobin, hemoglobin, globins, albumins, and some enzymes which are more water-soluble than other types of fish muscle proteins (Connell, 1980). An important issue in the seafood processing industry is proper fish species identification and some countries require listing the species on food packaging. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is used for species identification and sarcoplasmic proteins are used as the target proteins (An *et al.*, 1989).

Stroma proteins

Stroma proteins form connective tissue in the muscle structure. These proteins are completely water insoluble. They cannot be extracted in acid or alkaline solution, or physiological saline solution. The components of stroma proteins are collagen and elastin. Elastin is very resistant to moist heat and cooking does not affect elastin in the connective tissue. Dark fish meat contains more stroma proteins and fewer sarcoplasmic proteins than white fish meat.

6.3 Seafood proteins as a component of the human diet

6.3.1 Essential amino acids

All proteins, including those from fish, are chains of chemical units linked together to make a long and complex bio-molecule. These units, of which there are about twenty types, are called amino acids. Nine amino acids are generally regarded as essential nutrients for humans: histidine, phenylalanine, valine, threonine, tryptophan, leucine, isoleucine, methionine, and lysine. Two essential amino acids (EAA), lysine and methionine, are often referred to as limiting EAA. Lysine and methionine are typically found in high concentrations in seafood proteins, in contrast to, for example, cereal proteins (Taskaya *et al.*, 2009c; Chen *et al.*, 2007; 2009). Fish proteins provide a good combination of amino acids which meet human nutritional requirements and compare favorably with, for example, milk and soy proteins. Generally, EAA are found in slightly higher amounts in fish than shellfish, with freshwater species having a slightly higher content (Silva and Chamul, 2000). The biological value of seafood proteins is also high (FAO/WHO, 1990).

6.3.2 Non-essential amino acids

Non-essential amino acids (non-EAA) are amino acids that can be synthesized in the human body and unlike EAA, non-EAA do not have to be

provided in the diet. However, non-EAA are required for normal functioning of the human body. The non-EAA are alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine (Fennema, 1996).

6.3.3 Free amino acids and related compounds

The free amino acid (FAA) content in seafood muscle ranges from 0.5 to 2% of muscle weight. The FAA contribute to osmo-regulation and are depleted in fish muscle during starvation. Crustaceans such as lobster, shrimp, and crab have a higher content of FAA than finfish. Muscle tissue of aquacultured fish tends to have less FAA than wild fish. The content of non-protein nitrogenous (NPN) compounds in the white fish muscle makes up 9–15% of the total N, in clupeids 16–18%, and in some sharks up to 55%. The dark fish meat generally contains more NPN than the white fish meat. In the muscle tissue of mollusks and crustaceans, the NPN constitutes 20–25% of the total N. About 95% of the total amount of NPN in the muscle of marine fish and shellfish is composed of FAA, imidazole dipeptides, trimethylamine oxide (TMAO) and its degradation products, and betains (Ikeda, 1979).

6.4 Comparison of seafood proteins with vegetable and other animal proteins

Seafood is a highly nutritious food and its proteins significantly contribute to fulfill human nutritional needs. Muscle tissue of aquatic animals is particularly valuable for providing proteins of high quality that are considered nutritionally equivalent or slightly superior when compared to proteins derived from other animals, but of lower quality compared to egg proteins. This comparison is based on protein efficiency ratio, net protein utilization, and direct amino acid analysis. Also, it is generally accepted that the relative concentration of dietary EAA is the major factor determining the nutritional value of food protein. Proteins derived from aquatic and terrestrial animals are considered to be nutritionally superior to vegetable proteins because the content of dietary EAA is more complete in relation to human requirements. In addition, the biological value of proteins from aquatic and terrestrial animals is higher than that of vegetable proteins. Strong collagenous fibers and tendons are common in terrestrial animals, but are largely absent in seafood muscle. Therefore, seafood proteins are easily digestible and absorbable. In terms of animal nutrition and application of seafood proteins to animal feed stocks, the amino acids in fish meal are more digestible than vegetable proteins. As this is taken into account in the least-cost formation, it is likely to give higher value for fish meal relative to vegetable proteins than present formulations based on the content of total amino acids (Al-Kahtani *et al.*, 1998; Kristinsson and Rasco, 2000; Gopakumar,

2002; Venugopal, 2009). This presents a good practical opportunity for the utilization of seafood processing by-products if lipid-free protein fraction could be efficiently recovered. Although seafood lipids are abundant in omega-3 fatty acids, these fatty acids oxidize easily and impart fishy odor to meat of farm animals if the animals are fed excessive quantities. The nutritional value of proteins from different resources including seafood has been compiled by Friedman (1996).

It has been demonstrated that seafood has cardio-protective properties. In addition, fish consumption reduces plasma cholesterol levels, gives higher content of high-density lipoprotein (HDL), and alters the fatty acid composition in liver, plasma, and triglycerol-rich lipoproteins in the body. In contrast, diet based on proteins from terrestrial animals has been associated with various diseases in humans, which is due to the fact that food products rich in proteins derived from terrestrial animals generally have high content of saturated fat and dietary cholesterol. On the other hand, many studies report that vegetable proteins are associated with low blood cholesterol and low risk of the aforementioned diseases (Anonymous, 2010). Furthermore, vegetable proteins contain phytochemicals that contribute towards health and disease prevention.

6.5 Functional properties of seafood proteins

6.5.1 Solubility

Solubility of protein (or protein extractability) in salt solutions is one of the most important physicochemical properties in the manufacture of muscle food products. Protein solubility is often referred to as functional property or simply functionality. This characteristic is a result of comminuting and mixing of fish muscle tissue with salt. Protein extractability (solubility) is the percentage of total protein that enters the solution, but does not sediment due to centrifugation. High solubility of fish protein hydrolysates is often due to cleavage of proteins into smaller peptide units. These peptides typically have increased solubility mainly due to the reduction in molecular weight and increased number of polar groups available for hydrogen bonding with water dipoles. Increased solubility is not only due to smaller size, but also to the balance of hydrophilic and hydrophobic elements in the peptides (Shahidi and Botta, 1994). Besides NaCl, solubility of fish muscle proteins is also affected by pH. Protein solubility profiles (i.e., curves) are typically determined to establish conditions required to solubilize fish muscle proteins. Water solubility of sarcoplasmic and myofibrillar proteins as affected by pH and ionic strength (i.e., salt concentration) is shown in Fig. 6.4. The selective protein solubility by shifting pH and salt concentration of minced fish solution is a fundamental basis for the novel protein and lipid recovery technology taking advantage of isoelectric solubilization/precipitation (see Section 6.7).

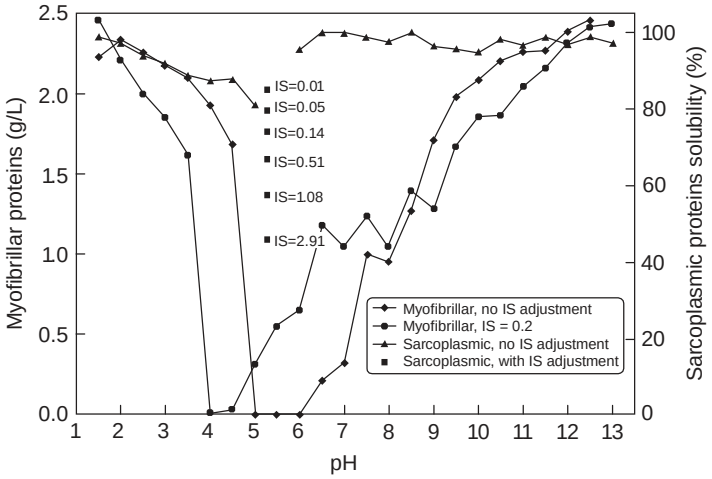


Fig. 6.4 Solubility of fish muscle proteins (i.e., myofibrillar and sarcoplasmic proteins) as affected by pH and ionic strength (adapted from Chen and Jaczynski, 2007b).

6.5.2 Gelation

Gel is an intermediate phase between a solid and a liquid. Technically, it is defined as a substantially diluted system which exhibits no steady flow. Protein gelation refers to transformation of protein from the sol state (viscous material) to a gel-like state (elastic material). When seafood muscle tissue is comminuted in the presence of NaCl, initially a viscous sol is formed, which after heating turns into a viscoelastic gel. The rheological properties of the gel depend on the characteristics of the myofibrillar proteins, which are affected by seafood species, freshness, pH, ionic strength, temperature, protein concentration, and others (Niwa, 1992). High ionic strength (i.e., added NaCl) favors myosin depolymerization resulting in increased surface hydrophobicity, even at temperatures below 30°C. The weak hydrophobic interactions between myosin molecules initiate formation of a gel structure. Myosin and actin are critical for thermal gelation of fish proteins. Myosin and actin undergo thermal denaturation at different temperatures, leading to the development of a typical gel texture. During thermal gelation of fish actomyosin complex, the viscosity increases between 30–41°C and 51–80°C (Sano *et al.*, 1988). The first increase corresponds to the endothermic transition of myosin, and the second to actin (Wright *et al.*, 1977). Muscle proteins isolated from cold-temperature fish such as Alaska pollack and hoki are known to gel at slightly above 0°C in a few hours due to endogenous enzyme in fish muscle, transglutaminase (Torley *et al.*, 1991). Gelation at low temperature due to transglutaminase is unique for seafood proteins and is often referred to as "suwari". The gel-forming

ability of dark fish muscle is lower than the white muscle due to the difference in unfolding ability of myosin rods.

6.5.3 Water-holding capacity

The ability of fish muscle proteins to hold water molecules during cooking and comminution is called water-holding capacity (WHC). The sensorial quality of final product such as tenderness, juiciness, or succulence is related to this functionality of fish muscle proteins. Decrease in WHC due to poor protein quality has a direct effect on moisture loss (i.e., drip) from a product and subsequently decrease in weight. Therefore, good WHC is desirable because it ultimately translates into the financial bottom line for fish processors. Fish muscle tissue consists of approximately 75% water which is hydrogen bonded by muscle proteins, mainly myosin and actin. Bound water accounts for about 4–5% of muscle water content and it firmly binds to the protein surface creating a hydration shell for the protein molecule. The 95% of the remaining muscle water content is called immobilized water and it is held by weak hydrogen bonds between muscle proteins and water dipoles.

6.5.4 Emulsification

Emulsion or emulsifying capacity is usually defined as the volume of oil that can be emulsified by protein before phase inversion or collapse of emulsion occurs. Stability of this system is facilitated by emulsifiers. Fish muscle proteins stabilize emulsions and are therefore good emulsifiers in food products. Emulsification of oil decreases surface tension and in turn decreases free energy, resulting in a thermodynamically more stable system. Emulsification of oil is possible because fish muscle proteins contain hydrophobic amino acids. The emulsification of oil with fish muscle proteins involves two steps: comminuting of fish muscle and heating. Fish muscle proteins have various emulsifying capacities and stabilize emulsions to different degrees (Table 6.2). Fish myosin and actin are the best emulsifiers. Protein hydrophobicity and length of protein chain influence this functionality (Gauthier *et al.*, 1993; Jost *et al.*, 1977).

Table 6.2 Stability of emulsions of salt soluble proteins (adapted from Wong, 1989)

Protein	pH	Ionic strength	Stability of emulsion (day)
Myosin	8	0.35	More than 4 weeks
Sarcoplasmic	7	0.35	12 hours
Actin-myosin	6.7	0.35	More than 3 weeks
Actin	7.2	0.35	Less than 36 hours

6.5.5 Whippability and foam stability

Foaming properties include whippability and foamibility. Both terms are used interchangeably in the literature. Foam consists of an aqueous continuous phase and gaseous dispersed phase. In most food products, proteins are the main surface-active agents that help in the formation and stabilization of the dispersed gas phase. Generally, protein-stabilized foams are formed by bubbling, whipping or shaking of a solution. Foaming properties of a protein refer to its ability to form a thin tenacious film at gas-liquid interface so that large quantities of gas bubbles can be incorporated and stabilized. Several studies have shown that protein-stabilized foams are most stable at the isoelectric point (pI) of protein, provided protein solubility is not affected. The pI of fish muscle proteins is at pH 5.5. NaCl at 0.5M enhances foaming of fish protein concentrate (FPC). The FPC with amphiphilic characteristics, that is with both hydrophobic and hydrophilic properties, has the best foaming properties. Foaming capacity of the FPC is provided by the soluble proteins (hydrophilic proteins), while the remaining denatured proteins (hydrophobic proteins) act as foam stabilizers (Fennema, 1996).

6.5.6 Effect of seafood protein functionalities on sensory quality of seafood products

Fish muscle proteins have unique functional properties, but due to the lack of a suitable purification process to preserve protein functionality, fish muscle proteins have been largely unavailable in the rapidly growing protein ingredient and health markets. Retaining the functional properties during the purification process is therefore of great importance. The sensory properties of food products results from interactions between several functional ingredients and proteins are one of the most important ingredients in food products. The physical and chemical properties that determine protein functionality include the size and the shape of the proteins, the charge and the distribution of charge and the flexibility as well as the ratio between the hydrophobicity and hydrophilicity (Rustad, 2007).

6.6 Factors affecting functional properties of seafood proteins

Proteins are the most abundant constituents of fish muscle and from the food processing point of view, probably the most important component. The composition and functional properties of fish muscle are influenced by several intrinsic (i.e., fish biology) and extrinsic (environmental) factors (Limin *et al.*, 2006; Özyurt and Polat, 2006; Abedian-Kenari *et al.*, 2009).

6.6.1 Intrinsic (biological) factors

Effect of seafood species and age

Fish species and age have a profound effect on the biochemical composition of fish. Generally, seafood contains 8–20% protein on wet weight basis. The wide range implicates that the protein content varies widely from species to species, among individual fish within a species, and also with age. The species with high protein content are shark, yellowtail, yellowfin tuna, and most salmonid species. Some other species with also relatively high protein content are Greenland halibut, Pacific herring, orange roughy, sablefish, sea trout, shad, and sturgeon. These fish average less than 16% protein, which is generally similar to chicken and beef. Most shellfish, including mollusks, have protein content similar to fish. Oysters and mussels contain less than 10% protein (Silva and Chamul, 2000).

Protein content of many fish species has been the subject of numerous investigations; but the relationship between the life stages (age) of fish and protein is not sufficiently known in the majority of species. Abedian-Kenari *et al*. (2009) reported that when Beluga sturgeons get older, the moisture content of the muscle decreases, while the protein content and total EAA increase. A general review of the effect of species and age on protein content of various animals including fish was provided by Clawson *et al*. (1991).

Effect of harvest seasonality

In the fish processing industry, seasonality of fish capture or the seasonality associated with fish growth in aquaculture systems is typical. As the season changes, the water temperature and available nutrients change. They are important parameters for fish protein composition and content. For example, wild sea bass has the highest protein (21.4–21.8%) and lowest moisture (70.8–71.0%) contents in spring/summer, when food availability is high; and the lowest protein (18.7–19.8%) and highest moisture (77.3–77.4%) contents in fall/winter (Özyurt and Polat, 2006). Sylvia *et al*. (1994) conducted a seasonal analysis of the proximate composition for Pacific whiting. Results showed that the lowest protein content with concurrent highest moisture content was in April. Sylvia *et al*. (1994) concluded that there is an inverse relationship between protein and moisture content. The same trend was confirmed by Hall and Ahmad (1997). In addition, Özyurt and Polat (2006) reported that the amount and type of amino acid in fish muscle were affected by season. Fish spawning and migration also affect protein content. Fish generally exhibit reduced meat yields, higher moisture and lower protein contents, and a loss of muscle integrity during spawning periods.

6.6.2 Extrinsic factors

Effect of harvest-to-processing time

Fish and fish products spoil more rapidly compared to most other muscle foods and, therefore, fish products are highly perishable. The spoilage begins

immediately following fish harvest. The rate of subsequent protein deterioration and consequently loss of functional properties is affected by capture method, handling, storage conditions, and most importantly time between harvest and processing.

Cold storage immediately following fish harvest is one of the most efficient ways to suppress spoilage and protein denaturation (Balachandran, 2001). The delay of chilling raw fish, even for a short time, typically accelerates deterioration of functional properties of fish muscle proteins (Karungi *et al*., 2004). Thus, after fish capture, it is extremely important to handle fish (washing, bleeding, and gutting) properly and as quickly as possible. Lowering fish temperature close to freezing delays the onset of rigor, proteolysis, in addition to extending the lag phase and growth of spoilage microorganisms.

Effect of storage on seafood proteins

Icing

Fish muscle proteins are similar to muscle proteins isolated from terrestrial animals in many respects. The postharvest handling of fish affects functional properties of proteins. Changes in fish muscle during storage directly affect sensory quality of food product derived from fish. In addition, enzymatic changes such as proteolysis of fish muscle influence the post-mortem deteriorative changes (Devadasan, 2002).

Seafood is usually stored under refrigeration or it is frozen. Icing is the most common preservation method employed onboard and in retail. It has been shown that fish muscle changes during ice storage and the rate of these changes varies with species, concentration of substrates and metabolites in the muscle tissue, microbial contamination, and conditions of storage after harvest (Simeonidou *et al*., 1997; Pacheco-Aguilar *et al*., 2000; Cakli *et al*., 2007; Özyurt *et al*., 2009). There is also a direct correlation between loss of freshness and the denaturation of fish myofibrillar proteins. Although the loss of functional properties is suppressed with icing, it is not eliminated. Holding fish in ice before mincing and freezing resulted in a significant loss of protein solubility, emulsifying capacity, water-binding capacity, cooking loss, thaw drip, and texture scores (Mohan *et al*., 2006).

In general, post-mortem ice storage for extended periods of time ultimately results in soft and unacceptable products (Devadasan, 2002). The complex changes causing fish muscle softening are mainly proteolysis by bacterial enzymes and autolysis by cathepsins, especially cathepsin L. On the other hand, icing has a small negative effect on the nutritive value of seafood. Melting ice removes some free amino acids and water-soluble proteins. If has been estimated that fish stored for 10–14 days in ice can lose up to 3% of the total proteins (Bramstedt, 1962).

Freezing

Freezing has largely been used to preserve fish sensory and nutritional quality. The most noticeable change in frozen fish is deterioration of

functional properties of proteins, texture scores, and thaw drip values as well as sensory attributes of cooked fish. The loss of functionality during frozen storage has been attributed to the intermolecular aggregation of proteins through hydrogen, hydrophobic, and disulfide bonds, resulting in loss of solubility, viscosity, and ATPase activity of myosin (Shenouda, 1980). Furthermore, during frozen storage, lipid oxidation occurs and its products facilitate protein denaturation (Sikorski and Kolakowska, 1994; Saeed and Howell, 2002).

Protein changes also affect flavor. Proteins in their native structure may bind undesirable flavors which are released during frozen storage. Many of the changes during frozen storage are related to the mechanism for how ice and ice crystals are formed. In addition, the unique enzymatic degradation of TMAO in frozen fish leads to changes in protein causing quality degradation. During TMAO degradation small amounts of formaldehyde are formed, inducing proteins aggregation, and thus reducing protein ability to bind water.

Although freezing is effective at inhibiting enzymes, enzymes in the fish muscle are still active at −17°C. Therefore, temperatures below −20°C are typically recommended for frozen storage of fish (Evans, 2008). Changes to fish proteins during freezing may be examined and monitored using proteome analysis. This is a relatively new technique for examination of the protein composition in cells and tissues such as fish muscle.

Effect of processing on seafood proteins

To increase shelf-life of fish during storage, some methods such as drying, salting, smoking, marinating, and canning have been used in the fish processing industry. A general objective of these techniques is to preserve fish food products by inhibiting microbial growth, while minimally changing or improving sensory quality. However, these processing techniques affect fish muscle proteins and therefore these changes are discussed separately in this section.

Emerging non-thermal methods (high-pressure processing and pulsed electric field)

Two new non-thermal preservation methods that cause minimal changes of sensory attributes are high-pressure processing (HPP) and high-intensity pulsed electric field (PEF). HPP processing extends shelf-life of seafood products by using extremely high pressure to non-thermally inactivate foodborne pathogens such as *Vibrios* ssp. HPP can have some applications in the surimi industry for development of novel products. Surimi forms a gel with excellent texture when exposed to heat in the presence of 2.0 to 2.5% salt. Instead of heat, pressure can be used to induce gelation of fish myofibrillar proteins in surimi. The pressure-initiated gelation may also be completed by mild heat. Alternatively, HPP-aided gelation could also be enhanced by transglutaminase (Ashie and Lanier, 1999). Pressure

treatment is also effective in producing highly appealing kamaboko from surimi. PEF applies mild electric current to food products to inactivate foodborne pathogens by electroporation of microbial cell membrane. There are very limited studies on the effects of PEF on fish muscle proteins. Based on SDS-PAGE, PEF processing does not affect fish muscle proteins (Gudmundsson and Hafsteinsson, 2001).

Irradiation

Ionizing radiation inactivates foodborne pathogens in fish products without heat; and therefore, it is often called "cold" pasteurization or sterilization depending on radiation dose (Jaczynski and Park, 2003a). Although irradiation is an effective means for extending shelf-life of fish products, indirect effects such as accelerated lipid oxidation, vitamin destruction, and some protein denaturation limits its application in fish processing (Ghadi and Venugopal, 1991; Al-Kahtani *et al*., 1998; Rahman, 1999). The effects of radiation on the fish muscle proteins have been studied relatively well and depend on the radiation dose (Al-Kahtani *et al*., 1998; Venugopal *et al*., 1998; Jaczynski and Park, 2003b; 2004). Proteins treated with ionizing energy are degraded into smaller molecules that upon digestion yield the same amino acids as the original proteins. Gamma radiation affects viscosity, solubility and stability of fish muscle proteins (Venugopal *et al*., 1998).

Drying (dehydration), salting, and smoking

Traditional fish preservation methods like drying, salting, and smoking influence overall nutritional value including protein composition. Drying may enhance oxidation and rancidity, and thereby cause a slight reduction in protein quality. The degree to which drying adversely affects protein quality depends on the drying temperature and time. Typically, the longer the drying process is, the greater protein degradation. When proteins are dried too fast, they harden (i.e., denature) resulting in a "crusty skin". When this happens water cannot escape from the core of fish muscle. The optimum drying temperature is 70–80°C or lower so that heat damage to protein quality is minimized. Drying at high temperatures such as 115°C or higher should be avoided because of its profound negative effects on protein quality (Ünlüsain *et al*., 2001). Control of time, temperature, and air flow are critical to maintain good quality of fish muscle protein during drying. Cold smoking has little effect on product composition (Silva and Chamul, 2000).

The main change caused by drying and smoking is water evaporation (i.e., dehydration), yielding increased protein and lipid content. Salting also causes dehydration by osmosis-driven plasmolysis, which similarly to drying involves dehydration of fish muscle. If the salt is ground too finely it causes fast withdrawal of moisture from the surface of fish muscle tissue resulting in a rapid protein denaturation leading to coagulation. Protein coagulation prevents further penetration of salt into the fish muscle causing a condition

called "salt burn". Salt-induced denaturation of fish muscle protein results in their decreased solubility.

Marinating

Marination is based on treatment of fish muscle with brine containing salt, spices, curds, lemon juice, etc. The main objective of marinating is to improve sensory quality and consumer acceptability of fish food products (Yashoda *et al*., 2005; Cutting, 1962). Fish marinating techniques and ingredients vary widely depending on the area of the world and culture. In the fish processing industry, the term ''marinated fish'' is used to define semi-preserved fish food products made by immersion on fish in a brine. The inhibitory effects of the brine on microbial growth and enzymes increase with brine concentration. It has been reported that marination enhances water-holding capacity, decreases cooking losses, and improves texture scores (Goodwin and Maness, 1984). However, depending on the marinating technique, marinated fish products may have a limited shelf-life (Fuselli *et al*., 1998). Fatty fish (5–15% fat) are typically used for marinating.

A considerable proteolysis occurs as a result of enzymatic activity during the "ripening" process. The soluble nitrogen increases due to the proteolysis of fish muscle protein to amino acids. In addition, actomyosin becomes less salt soluble. As a result of loss of protein and water, marinated fish typically have reduced weight. This loss can be as high as 40%. In addition, softening of fish bones due to vinegar increase calcium content.

Canning

Although canning of fish has little effect on proximate composition, some proteins are lost from fish muscle. The loss of proteins is due to pre-cooking, diffusion into the liquid phase, and heat destruction. Pre-cooking in steam or water is common in the fish canneries. During pre-cooking, some amino acids and water-soluble proteins are lost. Loss by diffusion into the liquid phase is of secondary importance because most fish muscle proteins coagulate when subjected to heat and therefore do not migrate into the liquid phase. However, the non-coagulating proteins diffuse into the liquid phase.

During heat-induced protein denaturation, the configuration of the native protein structure is altered, resulting in spatial rearrangement that leads to changes in functional properties. The denatured proteins coagulate resulting in their precipitation. This may affect protein digestibility and thus the nutritive value, even though there is no change in the amino acid composition.

Effect of cooking methods on seafood proteins

Cooking is applied to fish to enhance flavor, inactivate pathogens and increase shelf-life (Bognar, 1998; Türkkan *et al*., 2008). Cooking leads to denaturation of fish proteins due to the changes in protein conformation. Approximately 90% of fish proteins denature between 60 and 65°C, while

the remaining 10% (mainly tropomyosin) can withstand 100°C. The exact temperature at which denaturation takes place varies according to the type of protein and the presence of various protein types in different species. Typically differential scanning calorimetry (DSC) is employed to pinpoint the exact temperature for thermal transition of fish muscle proteins as well as the total energy required for that transition (i.e., denaturation) (Taskaya *et al*., 2009c). As a consequence of denaturation, proteins aggregate and form new bonds. The types of newly formed bonds, some of which are covalent, can be different from the original bonds (Opstvedt *et al*., 1984; Ünlüsain *et al*., 2001).

6.7 Isolation and recovery of fish muscle proteins from whole fish and fish processing by-products

In non-industrialized fisheries, most of the seafood resources were utilized for human consumption, animal feed, or plant fertilizer (Gildberg, 2002). However, mechanical processing of raw foods into food products generates by-products, and processing of aquatic animals is no exception. When fish are mechanically processed for fillets, the recovery yields are typically 30–40% of fillets and the by-products accounts for 60–70% by weight of whole fish (Torres *et al*., 2007). The by-products contain fish muscle proteins and omega-3-rich oil that could be recovered and used for human or animal consumption if a recovery technology is developed. In addition, low-value aquatic species such as Atlantic menhaden and arrowtooth flounder are not utilized for human consumption due to its bony and oily carcass characteristics. The isoelectric solubilization/precipitation (ISP) of muscle proteins with concurrent separation of lipids was proposed (Hultin and Kelleher, 1999, 2000, 2001, 2002). The ISP can be used to efficiently recover functional proteins from fish processing by-products and low-value aquatic species. While muscle proteins are in a soluble form, the insoluble components (bone, skins, scales, etc.) can be removed from solution by for example centrifugation, followed by protein precipitation at their isoelectric point (pH 5.5) and collection (Fig. 6.5). ISP allows efficient recovery of muscle proteins that retain gel forming ability and concurrent separation of omega-3-rich oil (Fig. 6.6). ISP processing in a continuous instead of batch mode has been applied to fish processing by-products (Chen and Jaczynski, 2007b; Chen *et al*., 2007), krill (Chen and Jaczynski, 2007a; Chen *et al*., 2009), and whole gutted carp (Taskaya *et al*., 2009a; 2009b).

Many published works have suggested that ISP processing of fish at basic pH allows recovery of proteins with generally better functionalities and, therefore, better thermally induced gelation and consequently texture as well as better color (i.e., whiteness) properties (Chen and Jaczynski, 2007a; 2007b; Nolsoe and Undeland, 2009; Taskaya *et al*., 2009a; 2009b). In addition, ISP at basic pH allows recovery of muscle proteins with higher nutritional

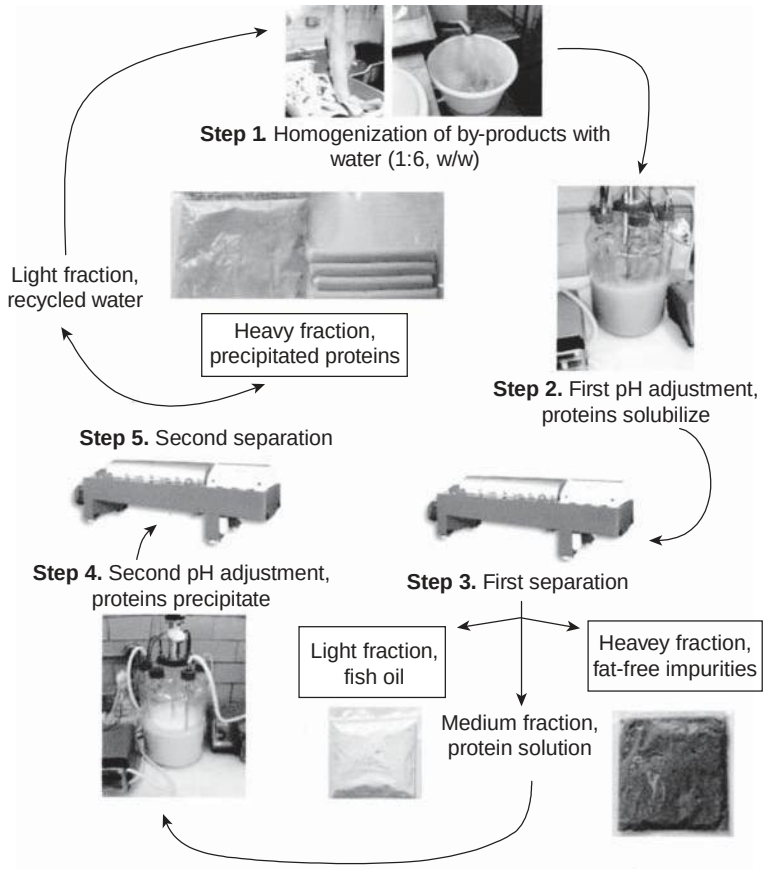


Fig. 6.5 Diagram of isoelectric solubilization/precipitation technology with concurrent oil separation proposed for recovery of muscle proteins and lipids from fish processing by-products and low-value aquatic animals. Materials in boxes are fractions to be further processed into food products and other applications.

quality as assessed by a greater content of essential amino acids (EAA) when compared to ISP at acidic pH (Chen *et al*., 2007; 2009). High quality protein (i.e., complete protein) is determined based on the presence of all nine essential amino acids (EAA) in adequate quantities to support human or animal health. When compared to the biological value (BV) of soybean protein concentrate and milk protein (casein), the BV for proteins recovered from fish processing by-products using ISP is higher than soybean protein concentrate and similar to milk protein. Egg protein is commonly referred to as a reference protein due to its high nutritional quality. Lysine is often considered a limiting EAA; therefore, it needs to be emphasized that proteins recovered from fish processing by-products with ISP had a

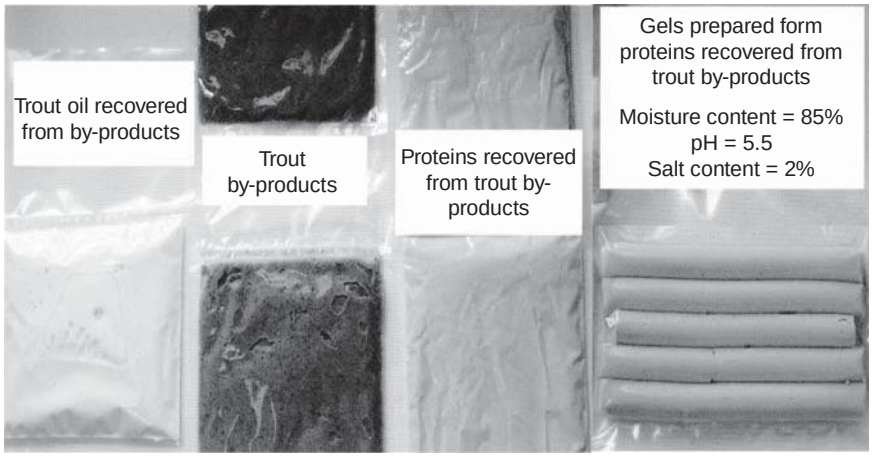


Fig. 6.6 Major materials recovered by isoelectric solubilization/precipitation technology from fish processing by-products are: (a) Fish oil recovered in Step 3 (Fig. 6.4). (b) Fat-free fraction containing bones, skin, scale, fin, insoluble proteins and others recovered in Step 3. (c) Fish muscle proteins recovered in Step 5. Gel-forming ability of proteins recovered in Step 5.

similar concentration of lysine as whole egg, and the concentration of lysine was even greater in proteins recovered from whole carp and whole krill (Taskaya *et al*., 2009c; Chen *et al*., 2009). Furthermore, due to extreme pH shifts during ISP, this technology shows some mild pasteurization effect in the recovered proteins, resulting in inactivation of *Escherichia coli* and *Listeria innocua* (Lansdowne *et al*., 2009a; 2009b). The biochemical principles of isoelectric behavior of fish muscle proteins are explained in Fig. 6.7.

6.8 Products derived from seafood proteins

6.8.1 Fermented seafood products

Fermentation has been considered as a traditional practice of producing various fish sauces and pastes in East and Southeast Asian (Lee *et al*., 1993). In general, fermented seafood products are prepared by salting followed by fermention (Shahidi, 2007). Fermentation is a microbial process under anaerobic conditions. Fermentation of fish also involves proteolysis by fish endogenous enzymes resulting in the production of some flavor compounds.

Fermented products such as fish sauce are composed of salt-soluble compounds derived from protein degradation, mainly free amino acids and peptides. Typically mixed cultures of salt-requiring (halophilic) and salt-tolerant (haloduric) bacteria are used in the fermentation process. The

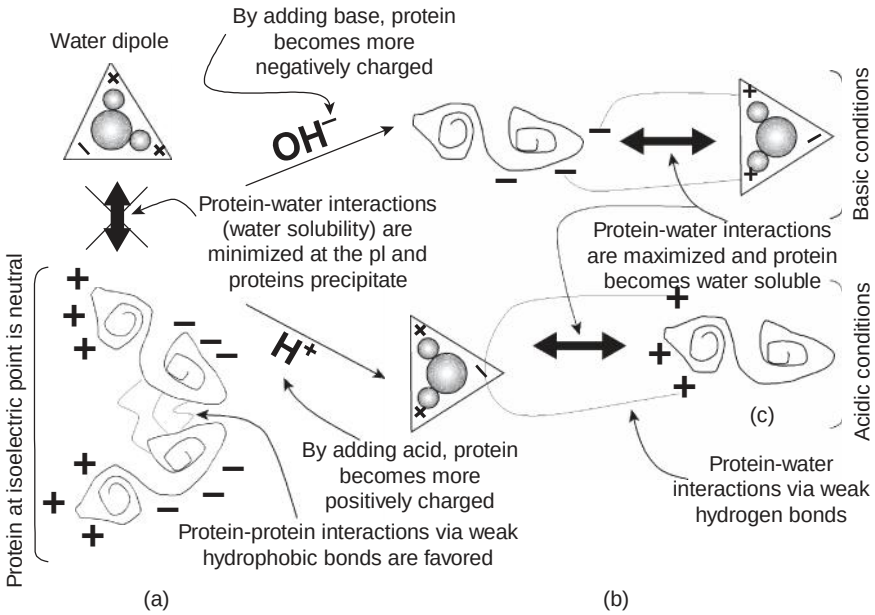


Fig. 6.7 A protein at its isoelectric point (pI) has a zero net electrostatic charge (adapted from Torres *et al.*, 2007). (a) At its pI, protein-water interactions are at the minimum, while protein-protein interactions via weak hydrophobic bonds are at the maximum, causing protein precipitation (i.e., lowest water solubility). (b) Protein-water interactions prevail under acidic or basic conditions when the pH of protein solution is adjusted away from the pI, resulting in protein water solubility.

aquatic species used for fermentation include anchovies, mackerel, lizard, clupeids, shad, and others. Freshwater fish are used for sauces such as *muoc-mam* and *mam-pla*. The main benefit of fermentation is enhancement of protein digestibility by making essential amino acids more available, especially lysine (Venugopal, 2009).

6.8.2 Seafood protein hydrolysates

One of the issues that the seafood industry currently faces is the amount of by-catch and processing by-products. These materials are rich in valuable and functional nutrients and they are considered a waste. At present, a large proportion of the captured seafood (~30%) is used for fish meal and animal feed. By developing enzyme technologies for protein recovery and modification, a production of food ingredients and industrial products may be possible. Enzymatic proteolysis can be applied to fish by-products resulting in the recovery of seafood protein hydrolysates (SPHs) (Kristinsson and Rasco, 2000).

SPHs have been considered as an alternative approach for converting underutilized fish biomass into edible protein products instead of animal feed or fertilizer. SPHs are prepared by digesting fish muscle tissue at the optimal temperature and pH for proteolytic enzymes such as papain, ficin, trypsin, pancreatin, pronase, or enzymes isolated from microorganisms. SPHs typically have high water solubility, high protein content, but low fat and ash contents. By using different fish species, enzymes, and digestion conditions, a wide range of SPHs with specific functionalities can be produced for different applications (Venugopal, 2006; 2009).

SPHs are used as protein supplements to cereal proteins such as those of wheat, rice, and corn. In addition, SPHs are used in dietetic foods as a source of small peptides and amino acids. The activity of these peptides depends on the raw material and hydrolysis conditions. SPHs at lower concentrations are used as cryoprotectants to prevent protein denaturation during frozen storage. The cryoprotective properties of SPH have been attributed to the stabilization of hydration shell surrounding myofibrils (Hoyle and Merritt, 2003). SPHs are also used in animal feeds and pet food, microbial growth media, as fertilizers, and as a source of novel bioactive peptides (Guérard, 2007; Venugopal, 2009).

6.8.3 Seafood protein powders

Tremendous quantities of fish processing by-products and low-value fish are discarded each year. Since they contain high-quality proteins, they are also used to manufacture seafood protein powders (SPP). Phillips *et al*. (1994) determined that protein-rich seafood processing by-products have a range of useful functional and nutritional properties and could potentially be used in food products. The SPPs are manufactured in a process that involves grinding, heating to denature enzymes and release lipids, sieving to remove bones and large tissue fragments, centrifugation to separate the proteins, followed by their dehydration and collection. Another method of extracting and concentrating proteins from seafood processing by-products involves using pH extraction and isoelectric precipitation (Choi and Park, 2002; Undeland *et al*., 2002; Kristinsson and Demir, 2003). SPPs have many applications in human food manufacturing, as animal feed ingredients, and in non-food industrial applications. SPPs are used as a dietary supplement added to various products to provide a source of easily digestible protein. SPPs are added to weight loss and protein supplements and are used as filler in encapsulated supplements.

An interest in bio-active SPPs for use as binders, emulsifiers, and gelling agents in food products has initiated renewed research in this field (Venugopal, 2009). A major effort was finalized in 1993 when the Association of Danish Fish Processing Industries and Exporters commercially manufactured fish-based SPP for use in frozen products to enhance water-binding and stability properties during frozen storage (Urch, 2001). More

recent developments resulted in SPPs from arrowtooth flounder and herring that displayed many desirable functional properties and contained high-quality proteins. Soluble SPPs were also developed from Alaska pollock processing by-products by the same researchers. These powders also had good functional, nutritional, and rheological properties (Sathivel *et al*., 2004).

6.8.4 Food coating films

The growing consumer demand for healthier food products and rising concerns about sustainability of natural resources has driven the development of new packaging systems that extend shelf-life and at the same time are recyclable. Some of the potential alternatives are edible coating films. These films are mainly derived from proteins, polysaccharides, and lipids (Fernández, 2006). Ideally, such films preserve food quality by restricting moisture loss and oxygen permeation, thereby reducing lipid oxidation, discoloration, and enhancing the overall organoleptic quality of food products. The films are also used as carriers to deliver and release antimicrobials and antioxidants (Iwata *et al*., 2000; Tanaka *et al*., 2001; Bourtoom, 2009).

Aquatic macromolecules such as fish myofibrillar proteins, collagen, and gelatin are good raw materials for the development of biodegradable and edible films. One of the methods of using low-cost fish, which are abundantly available, is to develop protein films that can enhance the storage quality of high-value fish products. A protein glaze for fish fillets or mince can be prepared from a portion of the same fish species, taking advantage of gel-forming properties of washed fish muscle (i.e., mainly myofibrillar proteins) in the presence of dilute organic acids such as acetic acid. This gives a natural protection to the fillet or mince. Casting of such films can make use of inherent properties of proteins, which include their ability to form gel networks, plasticity, and elasticity. The fish-based edible films can enhance the stability of frozen fish products. The major quality loss during frozen storage of fish is related to moisture loss from the muscle tissue leading to "freezer burn", lipid oxidation, and discoloration (Venugopal, 2009).

6.8.5 Injectable texturizer

Texturized proteins are defined as food products made from edible protein sources having structural integrity and identifiable texture. Proteins are conventionally texturized by various processes including fiber spinning thermoplastic extrusion techniques, curd formation, chewy gel formation, and film formation. Protein fibers are normally prepared from protein isolates containing 95–98% protein. The great potential of extrusion cooking to produce texturized protein from seafood, particularly underutilized

species and fish processing by-products is yet to be explored. Therefore, texturized seafood proteins may present a great future potential for commercialization and market success of seafood protein concentrates/isolates such as those recovered by isoelectric solubilization/precipitation (see Section 6.7). Under appropriate processing conditions texturization can improve protein functionalities while maintaining nutritional quality. The seafood protein concentrates/isolates have also been tested on a laboratory scale as a coating barrier for deep-fried food products to reduce fat uptake.

Most of the water in muscle is confined within the myofibrils in the spaces between the thick (myosin) and thin (actin) filaments. Therefore, any chemical, physical, or enzymatic means that increase the inter-filamental spaces can enhance water-binding potential in intact fish muscle, resulting in the entrapment of extraneous water. For example, seafood protein hydrolysates (SPH) (see Section 6.8.2) are more soluble than intact myofibrillar proteins. This is because upon hydrolysis proteins are broken down into peptides and free animo acids that exhibit increased water solubility compared to the parent protein (Mahmoud *et al*., 1992). The SPH are therefore added/injected (usually in a form of brine) to various food products. The increased water solubility and water binding of SPH has sparked interest in their use as injectable material to improve texture in seafood products and counteract softening of fish meat. The injection of brine containing SPH or homogenized muscle increases the weight gain in fish fillets by 5–20% and also increases cooking yield (Thorkelsson *et al*., 2008). The injection of SPH has been proposed as an alternative to application of phosphates in order to maintain moisture and prevent "drip" loss in seafood products.

6.8.6 Pet food and animal feed applications

It is generally accepted that the content of dietary essential amino acids is the major factor determining the nutritional value of food protein. This is why animal proteins are considered to be nutritionally superior to those from plants. Therefore, using fish protein isolates/concentrates and hydrolysates in animal feed stocks and pet food is highly feasible (Kristinsson and Rasco, 2000). Fish meal is by far the most important product currently made from fish by-catch, underutilized low-value fish, and seafood processing by-products. Fish meal is used in animal feed and pet food as a protein source. The quality of fish meal used for animal feed production depends on raw material and processing conditions, but normally it is a fine grayish/brown powder containing about 70% protein, 10% minerals, 9% fat, and 8% moisture (Gildberg, 2002). The expansion of aquaculture production in the past thirty years, and therefore, increased demand for aquacultured fish feed has put some economic stress on conventional uses of fish meal. As an alternative application that requires very good functional properties,

especially protein gelation, the proteins recovered from fish processing by-products with isoelectric solubilization/precipitation were used as a nutritive binder in pelletized livestock feed. Recovered proteins improved pellet quality, which has been correlated with increased animal growth performance (Gehring *et al*., 2009; Cutlip *et al*., 2008).

Following fish meal, fish silage is the second largest feed product made from underutilized low-value fish and fish processing by-product. It is typically regarded as an alternative to fish meal. Fish silage is used as a protein supplement in animal feed. This liquefied acid-preserved material is used directly as a feed ingredient. However, acidity and fat content limit the quantity of fish silage that can be fed to domestic animals. Although fish silage is a low-price product, it has a high nutritional value, and it is a valuable alternative for utilizing fish processing by-products which would otherwise be wasted.

6.8.7 Antioxidants

Oxidation of lipids initiates changes in food products which may affect flavor, color, and overall wholesomeness. The use of antioxidants is an effective way to minimize the oxidation process in foods (Hosseini *et al*., 2010). Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly added to food products. However, such compounds have been related to health risks resulting in consumer skepticism and strict regulations over their use in foods (McCarthy *et al*., 2001). Therefore, it is desirable to isolate antioxidants from natural sources.

Naturally occurring antioxidants can be found in a variety of marine organisms including fish, shellfish, invertebrates, and marine algae and bacteria. For instance, polyphenols and carotenoids are present in marine algae and micro-algae. Oysters and eel contain high levels of tocopherols. In addition, seafood protein hydrolysates (SPH) and chitosan exhibit antioxidant activity (Sathivel *et al*., 2003; Guérard *et al*., 2005). Shahidi *et al*. have conducted a number of studies on seafood-derived antioxidants. Shahidi *et al*. (1995) demonstrated that SPH effectively reduced lipid oxidation by up to 60% when added to pork products. Onodenalore and Shahidi (1998) showed that enzymatic extract of shrimp heads exhibited antioxidant activity in a model meat system. Hag fish and eel skin extracts contained heat-stable antioxidants and radical scavengers (Ekanayake *et al*., 2004). Tou *et al*. (2007) reported that Antarctic krill was an excellent source of astaxanthin, which has been attributed to high stability of omega-3-rich krill oil.

6.8.8 Antifreeze agents

Antifreeze proteins (AFPs) are proteins that have the ability to modify growth of ice crystals and depress the freezing point of water, resulting in

stabilization of ice crystals as well as inhibition of ice re-crystalization. Thus, AFPs improve quality of frozen fish products and maintain smooth texture. AFPs also reduce the temperature of chilling media used in fish storage and, therefore, prevent freezing of muscle tissue. AFPs are introduced into food products by physical processes such as mixing and soaking. Scholander *et al*. first reported AFPs in seafood (1957) that were later purified as antifreeze glycoproteins (DeVries and Wohlschlag, 1969). Depending on their amino acid content and structure, there are three main types of AFPs. However, all of them function similarly. The AFP-producing fish include Atlantic cod and Greenland cod, winter flounder, Atlantic wolf fish, and sculpins. AFPs from different fish species usually have similar structure. They are small proteins with a molecular weight of 3–26 kDa (Venugopal, 2006). They are usually purified from fish blood plasma or serum. Some of the seafood protein hydrolysates (SPH) display antifreeze properties. Addition of SPH containing 84–88% peptides during freezing of lizard fish resulted in increased volume of unfrozen water in myofibrillar proteins, resulting in less freeze-induced protein denaturation. The SPH peptides stabilized water molecules in the hydration shell surrounding myofibrillar proteins and therefore suppressed their denaturation (Venugopal, 2009).

6.8.9 Utilization of seafood enzymes

Enzymes are bioactive proteins that facilitate biochemical reactions in living organisms. Aquatic animals and plants contain the largest pool of diversified genetic material, and hence represent an enormous source of enzymes (Shahidi and Kamil, 2001). Enzymes can be isolated from aquatic animals and plants, but seafood processing by-products are a more economical source and, therefore, are more commonly used as a raw material for isolation of enzymes. Seafood enzymes are important industrial processing aids. Despite extensive research in marine enzyme technology, there are only a few successful applications in the food industry. A wider application of marine enzymes is limited due to the cost of recovery and competition from other sources. There is an increasing interest in marine enzymes as they show unique biological properties. Good reviews of marine enzyme technology have been provided by Díaz-López and García-Carreño (2000), Shahidi and Kamil (2001), and Morrissey and Okada (2007).

Marine enzymes are used in the production of fish protein hydrolysates, fermented fish products such as fish sauce, caviar production (for removal of the connective tissue surrounding eggs), fish silage, texture enhancement of surimi-based products (transglutaminase), cured fish products, and others. A common negative characteristic caused by proteolytic enzymes is softening of fish meat. To prevent meat softening, enzyme inhibitors are added during processing. A good example is surimi made from enzyme-laden Pacific whiting. Inhibitors are commonly added to Pacific whiting surimi to

suppress proteolysis of muscle proteins caused mainly by cathepsins that are believed to adhere to myofibrillar proteins (Klesk *et al.*, 2000; Park and Lanier, 2000). Marine enzymes are also applied in dairy processing. In addition, other applications have been suggested such as in tenderization of meat by fish collagenases and clarification of fruit juice.

6.8.10 Seaweed protein

Seaweed is a major group of aquatic food products that are traditionally used in human and animal nutrition. The world aquatic plant production by aquaculture was 15.1 million tons in 2006. The culture of aquatic plants has been consistently increasing, with an average annual growth of 8% since 1970 (FAO, 2009). Generally, the protein content of marine algae is low for brown seaweeds (3–15% of dry weight), moderate for green algae (9–26%) and high for red seaweeds (up to 47%). For most seaweed species, major amino acids are aspartic and glutamic acids (Fleurence, 1999; 2004). Numerous seaweeds that are rich in proteins are used in food preparation in different cuisines. The best known example is *Porphyra* used as a sushi wrap (Munda, 1977). Green seaweeds such as *Spirulina* containing moderate protein content are also processed for various food products.

6.9 Environmental considerations for continuous sustainability of proteins from aquatic resources

Proteins derived from aquatic resources are gaining popularity worldwide as indicated by increased per capita consumption (Table 6.1). The increased demand for traditional raw materials is leading to significant pressure on current aquatic resources. Although conventional marine resources are being gradually depleted of some species, aquaculture production has become an important source of seafood providing a stopgap for the dwindling marine resources (Table 6.1). The decline in some of the capture fisheries is likely to have a serious impact on food security and economy in the developing countries (Venugopal, 2006). Over-fishing will likely change ocean environmental dynamics translating into profound global effects. Therefore, there is an urgent need to control environmental stress in the marine environment by concerted international regulations in order to maintain sustainable production of proteins from aquatic resources.

The world population has been forecast to increase to 8.5 billion in the next 25 years. In order to meet global demand for aquatic proteins, the production will have to increase accordingly. While efforts are needed to maintain sustainable fish production to satisfy the demand, the higher fish supply from the capture fisheries in unlikely (FAO, 2009). However, Table 6.1 shows that about 92 million tons of fish are captured every year, of which 33 million tons are transformed into non-food uses. In addition, the amounts

of seafood processing by-products and fish by-catch are staggering. Therefore, to meet the increasing demand for aquatic proteins, it will be critical to utilize our raw materials more efficiently.

6.10 Regulatory aspects of seafood protein: allergies to seafood proteins

Seafood plays an important role in the human diet, providing a valuable source of high quality protein. At the same time, the protein is among the most common causes of food allergies and consumers should be aware of this issue. To address this problem from a regulatory standpoint and to protect consumers in the United States, the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) (Public Law 108-282) was enacted in August 2004. This act specifies labeling requirements for food products that contain food allergens. According to FALCPA, all packaged food products regulated under the Federal Food, Drug, and Cosmetic Act (FFD&C Act) that are labeled on or after January 1, 2006, must comply with FALCPA's food allergen labeling requirements. Under FALCPA, crustacean shellfish (such as crab, lobster, and shrimp) and ingredients that contain protein derived from crustaceans are major food allergens, but molluscan shellfish (such as oysters, clams, mussels, and scallops) are not (FDA, 2009).

Most of the consumers allergic to seafood do not tolerate cod. Therefore, cod is usually used as a reference to which other seafood allergens are related. The allergenic properties of cod proteins have been extensively studied. The major allergen of cod proteins is *Gad c1* (allergen M), which is a parvalbumin (Aas and Jebsen, 1967; Elsayed *et al*., 1972; Apold and Elsayed, 1979a; 1979b; 1980). Fish muscle parvalbumin is a stable, acidic, Ca-binding protein (12 kDa) that is resistant to heat and chemical denaturation, as well as enzymatic proteolysis (Aas and Elsayed, 1969; Elsayed and Aas, 1971; Elsayed and Apold, 1983). Parvalbumins are present in relatively high amounts in white muscles of lower vertebrates and in lower amounts in fast twitch muscles of higher vertebrates (Lehky *et al*., 1974). It has been demonstrated that parvalbumin is present in white muscle of many seafood species (Aas, 1987; Elsayed and Apold, 1983). The clinical symptoms related to allergies induced by seafood proteins might be manifested in a variety of symptoms such as urticaria, allergic contact dermatitis, rhinoconjunctivitis, asthma, oral allergy syndrome, diarrhea, or anaphylaxis.

In the European Union there is a comparable act addressing the same issue of food allergies associated with seafood consumption. This act is titled: "Food Labelling (Declaration of Allergens) Regulations 2009 (S.I. No. 2801 of 2009)". This act is an amendment of the Food Labeling Regulations from 1996 with respect to the labeling requirements for food products containing known allergenic ingredients, including labeling exemptions for certain processed forms of those ingredients.

6.11 References

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7

Egg proteins

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Abstract: This chapter investigates the uses of hen egg white and yolk proteins in food processing. Egg whites are used extensively as ingredients in food processing due to their unique functional properties such as foaming and gelling. Without the existence of a complex macrostructure, egg white is more or less a colloidal suspension of different protein constituents. The egg white proteins represent more than 80% of the total dry matter in egg white. Therefore investigations into the physicochemical characteristics, e.g. the isoelectric point of these proteins, have promoted the elucidation of their structure-function relationships for the benefit of food processing. Egg yolk, meanwhile, is an essential ingredient in the preparation of a large variety of food emulsions. The potential of egg yolk has not been sufficiently explored however, so many research activities, such as pre-processing by a variation of the environmental conditions, heat treatment and enzymatic modification via phospholipase A_2, are now focussing on the development of new methods to improve the technological functionality of egg yolk.

Key words: egg yolk, egg white, ionic strength, gelation, ovalbumin, ovotransferrin, lysozyme, air–water interface, protein denaturation, LDL, emulsifying properties, phospholipase.

7.1 Introduction

Egg processing is a way to preserve the egg, to delay its consumption, to facilitate its transportation and its incorporation in manufactured food products. Therefore hen egg is one of the most versatile foods. It offers a high-quality protein resource as well as an important content of lipids, valuable minerals, carbohydrates, and vitamins. In the food industry, egg products are used for their nutritional value and organoleptic characteristics, but also and mainly for their functional properties.

Of course, the primary aim of the laying hen is not to produce high-value human food but to give rise to new life. Therefore, avian eggs contain the

basic elements for life, and many of the egg compounds have so-called biological activity. For example, almost all the albumen proteins are antimicrobial, thus protecting the developing embryo. Consequently, hen eggs are very good potential sources of raw materials for health-promoting, so-called functional foods, as well as for the traditional food industries. No other ingredient possesses the same combination of functional properties, making it difficult to substitute egg products in many food preparations. Both egg yolk and egg white have very good coagulation properties, particularly when exposed to heat. These are sought after for products which are cooked, typically in pastry making (e.g. cakes, flans, and creams) and meat products (e.g. sausages, surimi). Egg white alone is renowned for its excellent foaming properties, essential in desserts, cakes, biscuits and many aerated prepared dishes like soufflé and mousse. Egg yolk is recognised for its emulsifying properties, of particular value for the preparation of hot and cold sauces (e.g. mayonnaise, dressings, hollandaise sauce), but also in the preparation of biscuits, cakes and ice cream.

In order to meet the increasing demand for egg products from the food industry, egg processors have developed techniques to preserve egg white and egg yolk after separation. The most commonly used are drying and pasteurising. Besides these techniques the egg processing industry is searching for new methods to improve the technological functionality of egg products. Such a functionalising through pre-processing includes enzymatic and heat treatment as well as variations of environmental conditions. To combine the requirements for preservation and functionalising, a detailed knowledge of the structure-function relationship of all egg constituents is necessary. For example, egg processors and users would prefer to benefit from a longer shelf life and improved microbiological safety by applying more intense heat treatment to liquid egg yolk. However, existing differences in the pasteurisation intensity between egg product manufacturers lead to difficulties for the food emulsion manufacturers in controlling the properties of final products. This gap in knowledge is ascribable to the extreme complexity of the chemical composition of egg components, and the fact that the unique properties are partly driven by complex molecular interactions.

To cope with these difficulties, this chapter will present a synthetic view of the state of knowledge regarding the structure of egg components with particular emphasis on proteins. The chapter begins by detailing the chemical characteristics and application of egg white components. We focus on the foaming and gelling properties of egg white as well as possible interactions with polysaccharide food ingredients. We pay particular attention to updating the data, notably by the contribution of our own research results. The second half of the chapter contains information concerning the structure, composition, extraction and properties of egg yolk. We then review the current understanding of egg yolk properties, interactions between constituents and possible applications due to different manufacturing steps.

Additionally attention is paid to the separation of egg yolk into its main fractions: egg yolk, granula and plasma.

7.2 Egg white: chemical composition and structure

The solid concentration of liquid egg white is usually close to 12%. The variation within the dry matter content should be less then 1%. An increase in the solid content of liquid egg white is observable if eggs are used that have lost considerable moisture, for example those with large air cells or due to long storage times. To decrease the solid level, fresh eggs from older birds have to be used. Also hot weather decreases the egg white solid level. This seasonal variation has to be considered especially for eggs from free range hens. The pH of egg white can vary from 7.6 to 9.3, but the most common is between 8.4 and 9.2. Most commercial liquid egg white has a pH of 9.1 ± 0.1. The increase is strictly a function of the amount of carbon dioxide lost from the egg white. Its rate of loss depends on the temperature of the egg, the amount of carbon dioxide in the environment, and the degree of shell sealing (Stadelmann and Cotterill, 1986).

Tables 7.1 and 7.2 give an overview of the major constituents of egg white as well as some of their physicochemical and functionally important characteristics (Tilgner, 2009). Protein (albumen) is the major component of egg white with an average amount of 9.7% to 10.6% (w/w). The carbohydrates account for only 0.5–0.6% of the egg white dry matter, where glucose represents, with 98%, the majority of free carbohydrates (Mine, 1995). About 55% of the total amount of carbohydrates is combined to protein structures (Ternes, 2008). The amount of lipids (0.01%) in egg white is negligible compared with egg yolk. It is reported by Ternes (2008) that egg white has no specific macrostructure as it is known from the egg yolk. Only one protein constituent, ovomucin, is thought to be responsible for the gel-like properties of fresh thick egg albumen. During storage, egg white thinning,

Table 7.1 Composition and physicochemical properties of the major egg white proteins (Tilgner, 2009)

Protein fraction	Rel. amount [%]	SH/SS [–]	MG [kDa]	IEP [–]	Glycosylated [–]
Ovalbumin	54	4/2	45	4.6–4.8	✓
Ovotransferrin	12–13	0/15	76–77.7	6.1–6.6	✓
Ovomucoid	11	0/9	28	4.1	✓
G2 Globulin	4.0	–	40–49	5.5	✓
G3 Globulin	4.0	–	49–58	4.8–5.8	✓
Lysozym	3.4–3.5	0/4	14.3	10.7	–
Ovomucin	1.5–3.5	–	230–8300	4.5–5.0	✓

Table 7.2 Denaturation temperature T_D [°C] for the major egg white proteins in the native and isolated status measured by differential scanning calorimetry (Tilgner, 2009)

	Denaturation temperature T_D [°C]			
	In the native form (egg white)		In the isolated form	
Protein fraction	*pH 7*	*pH 9*	*pH 7*	*pH 9*
Ovalbumin	84.5	84.0	84.0	84.0
Ovotransferrin	65.0	69.5	61.0	62.0
Al^{3+} Ovotransferrin	76.5	–	73.5	72.5
Ovomucoid	–	–	79.0	79.0
Lysozym	74.0	–	75.0	72.5
Globuline	–	–	92.5	–

an important change in egg white occurs (Hammershøj *et al*., 2002). It is usually attributed to the degradation of the ovomucin complex (Kato *et al*., 1971; Robinson and Monsey, 1972). Many theories have been suggested to explain egg white thinning, but there seems to be no general consensus among researchers about the causal factors that induce this phenomenon (Huopalahti *et al*., 2007).

7.2.1 Constituents of egg white

Without the existence of a complex macrostructure, egg white is more or less a colloidal suspension of different protein constituents. To give a short overview of the protein composition and its important characteristics, the following section deals with the main compounds of egg white. The focus is on the techno-functional properties, whereas a detailed description of the bioactive and pharmaceutical applications can be found in Huopalahti *et al*. (2007).

Ovalbumin

Ovalbumin is the predominant protein in albumen and represents 54% to 58% of the egg white protein by weight. It is a monomeric phosphoglycoprotein with a molecular weight of 44.5 kDa and an isoelectric point (IEP) of 4.5. Ovalbumin is the only egg white protein to contain free sulfhydryl groups. The complete amino acid sequence of hen ovalbumin comprises 385 residues. The purified form of ovalbumin consists of three different subclasses, Al, A2 and A3, which contain two, one and no phosphate groups per molecule, respectively (Mine, 1995). During the storage of eggs the ovalbumin is converted to S-ovalbumin, which is a more heat-stable form. This mechanism was primarily described by Smith and Back (1965) and

Donovan and Mapes (1976). The authors found that the denaturation temperature of ovalbumin was shifted from 84.5 °C to 92.5 °C for S-ovalbumin, with an intermediate form denatured at 88.5 °C. In fresh laid egg white about 5% of ovalbumin exists in the S-form, while more than 50% of the ovalbumin converts to the S species by the time eggs reach the supermarket and eventually the consumer (Hammershøj *et al*., 2002). It is reported that the percentage of S-ovalbumin reaches 81% after 6 months storage at low temperature (Huopalahti *et al*., 2007). Eggs with high S-ovalbumin content have runny whites and do not congeal as effectively on cooking. Furthermore, the conversion rate increases with high storage temperature (50 °C) and increasing pH. S-ovalbumin is easily formed *in vitro* by a 20 h incubation at 55 °C in 100 mM sodium phosphate at pH of 10 (Hammershøj *et al*., 2002). A high pH and temperature increase the rate of conversion. Particularly with regard to food applications, the conversion of ovalbumin in the S-form has to be accounted for.

Ovotransferrin

Ovotransferrin (formally Conalbumin) represents 13% of the total egg white protein content. It is a glycoprotein and responsible for the transfer of ferric ions from the hen's oviduct to the developing embryo (Huopalahti *et al*., 2007). It has been demonstrated by Mason *et al*. (1996) that ovotransferrin has the ability of binding two Fe^{3+} ions per molecule with a high affinity. The IEP of ovotransferrin depends on the quantity of fixed Fe^{3+} ions and varies between pH 7.2, pH 6.6 and pH 6.1 for no, one and two bound ferric ions, respectively. The denaturation temperature of ovotranferrin can be increased from 63 °C to 83.5 °C due to the complexation of Fe^{3+} at pH of 7.5 (Ternes, 2008). It has to be mentioned that due to fixation of Fe^{3+} ions accompanying red colour changes occur in the egg white. However, the use of aluminium salts might be promising because of their ability to improve stability without producing negative colour effects (Mine, 1995). It is known from studies by Giansanti *et al*. (2002, 2005) that ovotransferrin has a good antimicrobial and antiviral effect. Additionally it is suggested by Huopalahti *et al*. (2007) that ovotransferrin can be used as a nutritional ingredient in iron-fortified products such as iron supplements, iron-fortified mixes for instant drinks, sports bars, protein supplements and iron-fortified beverages.

Ovomucoid

Ovomucoid, a glycoprotein, represents 11% of the total protein content in egg white and shows a trypsin inhibitory activity. Due to this enzyme inhibition function (ovomucoid has nine disulfide bridges in its structure) ovomucoid is very stable against digestive enzymes and high temperatures. It can be heated at 100 °C under acidic conditions for long periods without any significant changes in its physical or chemical properties. Ovomucoid has an IEP between pH 3.8 and 4.4 depending on the attached glycosyl

residues (Ternes, 2008). A large proportion of the carbohydrates (25%) present in this glycoprotein are joined to the polypeptide chain through asparaginyl residues (Mine, 1995).

Ovomucin

Ovomucin is a sulphated egg white glycoprotein with a highly viscous and gel-like structure. Ovomucin represents about 3.5% of the total egg albumen protein and its content in thick egg white is four times higher than in thin white (Ternes, 2008). It is characterised by high molecular weight and a subunit structure of α - and β -forms.

The molecular weight of the α -ovomucin subunit has been estimated by SDS-PAGE or ultracentrifugation to be between 180 and 220 kDa and for β -ovomucin between 400 and 720 kDa (Huopalahti *et al*., 2007). As already mentioned, ovomucin is responsible for the gel-like structure of fresh egg white. It forms an interlinked network of mucin fibrillae with a diameter of
2–10 nm to prevent bacterial movement within the egg white. The fibril structure is disintegrated during storage of the egg. The carbohydrate contents of α -ovomucin and β -ovomucin are roughly 15% and 60%, respectively. The unfractionated ovomucin consists of 10–12% hexosamine, 15% hexose and
8% sialic acid. Ovomucin and ovomucin-derived peptides are known to possess different kinds of biological activities. As described by Yokota *et al*. (1999), β -ovomucin shows growth-inhibiting and cell-damaging effects on sarcoma cells. Additionally it is shown that ovomucin has antitumour effects (Watanabe *et al*., 1998a) and hemagglutination inhibition activity against bovine rotavirus, hen Newcastle disease virus, and human influenza virus (Watanabe *et al*., 1998b). Besides the demand for a nutritional and func- tional value of proteins, there is a growing interest in health-promoting addi- tives. Due to its pharmaceutical benefits ovomucin seems to be a good potential source of bioactive ingredients for novel functional foods.

Lysozyme

Lysozyme, also known as muramidase or N-acetylmuramichydrolase, represents 3.5% of the total egg white protein content. Hen egg white is the most commercial source of lysozyme with a concentration of 3500 μ g/ml. Lysozyme is a relatively small secretory enzyme with a molecular mass of
14.3 kDa and no carbohydrate compounds. It is a very basic protein with an IEP at pH 10.5. Due to this high IEP value the enzyme interacts with ovomucin (IEP 4.5–5.0) and negatively charged residues of sialic acid in glycoprotein, as well as ovotransferin (IEP 6.1) and ovalbumin (IEP 4.5). Lysozyme catalyses the hydrolysis of the β -1-4 glycosidic bond of specific polysaccharides contained in cell walls of bacteria. It shows bacteriostatic, bacteriolytic, and bacteriocidal activity, especially against Gram-positive bacteria. This characteristic has found several applications in the food and

pharmaceutical industries. Appendini and Hotchkiss (1997) incorporated an active form of lysozyme into food packaging materials to extend the

shelf life of non-sterile or minimally processed foods by preventing the contamination by or growth of microorganisms.

To extend the shelf life of poultry meat under refrigerated storage Kijowski *et al*. (2005) suggested that a treatment with lysozyme solution could be used as an effective antimicrobial. In cheese making, lysozyme has been used to prevent growth of *Clostridium tyrobutyricum*, which causes off flavours and unwanted fermentation in some cheeses. To broaden the spectra of possible applications lysozyme has undertaken several chemical and thermal modifications. Heat denaturation of lysozyme results in the progressive loss of enzymatic activity, but a greatly improved antimicrobial action towards Gram-negative bacteria. As reported by Lesnierowski *et al*. (2004), the combined thermal and chemical modifications lead to the formation of an enzyme preparation with increased content of polymeric forms which include lysozyme activity to Gram-negative bacteria.

Globulin

G_2 and G_3 globulin each represent about 4% of the total protein content in egg white. They have an IEP at pH 4.8 to 5.8 depending on the amount of linked carbohydrates (Weijer *et al*., 2006). Analyses of amino acid and carbohydrate composition (3.2–3.7% hexose, 2.4–2.5% hexosamine) showed a high similarity of both proteins (Mine, 1995). Furthermore, the molecular weights of G_2 and G_3 globulins were roughly estimated to be 49.0 kDa (Weijer *et al*., 2006). In contrast to the G_1 globulin, which was identified as lysozyme, there is only little reported to G_2 and G_3. From a techno-functional point of view it is reported by Ternes (2008) and Mine (1995) that the globulins play an important role for the foaming properties of egg white. The authors stated that the foam stability as well as the foam overrun is positively influenced by the presence of globulins.

7.3 Manufacture of egg white ingredients

Spray-dried egg white is commonly used as a food ingredient for its foaming and gelling properties. Additionally the removal of water to a low enough level stops the growth of microorganisms and slows chemical reactions. Thus, dehydration is a successful way of preserving egg white. There are a number of different types of dried egg white products available. The most important types of commercial dried egg white products are spray-dried and pan-dried products; the spray-dried egg white is also available in an instant-dissolving form. Agglomerated instant egg white provides good dispersing characteristics and rapid dissolving properties when added to water. Most dried egg white products are available in a whipping or non-whipping type, depending on the functional properties required. For example, there is demand for an excellent whipping of dried egg white for use in biscuit, cakes and meringues. On the other hand, there are several uses where the

whipping properties are not necessary but the demand for excellent gelling properties dominates. It has to be mentioned that most commercially available whipping egg white powders have a whipping aid, such as sodium laryl sulfate, triethyl citrate, xanthan gum or sodium oleate. These additives are used at about 0.1% based on the egg white solids and depending on the type of additives. Depending on the regulatory status of each country, it is necessary to declare the addition of such additives in the final product. Pan-dried egg white represents only a small branch and is mostly used for the production of aerated confectioneries. Pan drying produces flake type material to a moisture level of about 14% (Stadelmann and Cotterill, 1986). Due to the heat sensitivity of egg white proteins the temperature control of all drying methods is very important. It is necessary to preserve the native characteristics of egg white proteins, which includes the ability to gel with heat and the production of stable foams.

Lechevalier *et al*. (2007) analysed the influence of industrial processing steps during egg white drying on the protein structure and functionalities. The authors concluded that the most critical step was the spray drying that strongly damaged foaming properties. During this step, heat transfers and the air–product interface are more responsible than shear rates for the negative changes occurred in egg white foaming properties. In most cases of industrial processing, the air inlet temperature is set higher than 140 °C. Because of heat sensitivity of egg white proteins, Ayadi *et al*. (2008) tested moderate drying conditions with low air inlet temperature <120 °C and short residence time in the dryer. Their results showed that spray drying under moderate scale led to egg products with good foaming, gelling and emulsifying properties.

Nevertheless Lechevalier *et al*. (2005b) highlighted that heat treatment is not the only critical point of the process. In their study they showed a negative influence on the foaming properties of shear rates and stainless steel–product interfaces during tank storage, pasteurisation and homogenisation. To overcome the negative effects of spray drying it is suggested by several authors (Lechevalier *et al*., 2007; Hammershøj *et al*., 2004, 2006a, 2006b) to combine the egg white processing with heat treatment of the dry egg white powder.

Due to its importance in egg white processing, this technology will be discussed separately later on. Additionally, Lechevalier *et al*. (2007) highlighted the fact that a desugarisation step before spray drying had an improving effect on egg white foaming properties.

7.4 Functional properties of egg white

7.4.1 Gelling properties of egg white proteins

The success of many cooked or baked food products is dependent on the coagulation of proteins, especially the irreversible heat coagulation of egg

white proteins. The loss of fluidity of liquid egg white is detectable at 60 °C. Donovan *et al*. (1975) measured the thermal denaturation by differential scanning calorimetry. It is reported by the authors that the thermogram of egg white shows three major endotherms at 60 to 65 °C (ovotransferrin), 70 to 75 °C (lysozyme) and 80 to 85 °C (ovalbumin). Ovotransferrin is the most easily heat-denatured egg white protein. Therefore, the protein plays a major role in the initiation of egg white coagulation. However, the complexation of copper and aluminum increase heat stability and maintain the functionality of ovotransferrin (Mine, 1995).

The denaturation of proteins involves the breakage of hydrogen bonds, the uncoiling of polypeptide chains and the exposure of reactive groups. A scheme for the thermocoagulation of egg white, highlighting the participation of the disulfide bonds, the protein net charge, and the intermolecular hydrophobic interactions, was proposed by Ma and Holme (1982) and Mine (1995). The authors described the following mechanism for the heat-induced coagulation of egg white. After the initial unfolding of the native protein, the formation of spherical aggregates via hydrophobic interactions occurs. This first step leads to turbidity in the liquid egg white. The second step includes the stiffening of the preformed aggregates through sulfhydryl-disulfide reactions. With the participation of sulfhydryl-disulfide interchanges, the pre-formed aggregates subsequently thicken and set to a gel or coagulum (Mine 1995; Croguennec *et al*., 2002). During the cooling stage, the rheological properties of egg white gels increase suddenly by formation of numerous hydrogen bonds. Additionally it has been reported that the formation of stable intermolecular β-sheet structures plays the major role during aggregation of egg white. Ovalbumin, ovotransferrin and lysozyme form these intermolecular β-sheets. During the β-sheet transition, the functional groups that are engaged in intramolecular hydrogen bonding, electrostatic and hydrophobic interactions in the native state, become available for intermolecular interactions forming a gel network (Mine, 1995; Tilgner,
2009). It has to be mentioned that lysozyme undergoes a thermal denaturation with a β-sheet formation but did not gel when heated at 80 °C nor at 95 °C in an isolated status (Mine, 1995). Therefore the functional role of lysozyme in the egg white gelation process remains as yet unclear. However, an important fact that has to be considered in this context is the IEP of the main egg white proteins ovalbumin, ovotransferrin and lysozyme. Ovalbumin has the more acid IEP of pH 4.5, ovotransferrin is quasi neutral with an IEP at pH 6.5, and lysozyme has the highest IEP at pH 10.5, which makes it the only egg white protein that is positively charged in physiological conditions (Lechevalier *et al*., 2003). An electrostatic complex formation between lysozyme and other egg white proteins as described for the competitive adsorption at the air–water interface might be possible (Damodaran *et al*., 1998).

The conditions for forming a gel or coagulum depend on the availability of reaction partners and therefore on the relative speeds for unfolding of

proteins (Croguennec *et al.*, 2002). In this context the partial unfolded reactive state of a protein, also known as molten globule state, seems to play a major role in the molecular interactions. A new method for the formation of the molten intermediate structure is represented by a controlled heating of dry egg white powder (Hammershøj *et al.*, 2006a, 2006b). This promising technology will be discussed in Section 7.4.4.

7.4.2 Influence of pH and salts on the gelling properties of egg white proteins

Egg white gels consist of a connected three-dimensional network. It is reported by Croguennec *et al.* (2002) that the properties of the network depend mainly on the physicochemical conditions of the medium, especially on the pH, ionic strength and type of salts. A comparison of the gel rigidity and the water-holding capacity of different egg white gels showed that both characteristics had the best performance for translucent and semi-translucent gels (Doi and Kitabatake, 1997).

Ovalbumin is known to have a predominant role in the formation of egg white gels. Hatta *et al.* (1986) showed the importance of pH and ionic strength on the turbidity and hardness of heat-induced ovalbumin gels. Depending on the environmental conditions in the medium, a transparent solution, transparent gel, turbid gel or turbid solution could be obtained due to a heating step. The gel hardness was maximal under conditions that lead to a transparent or slightly turbid gel. The authors measured higher gel strength for the transparent or slightly turbid gels formed at pH 3.5 and pH 6.5 or pH 7.0, respectively. The highly turbid gel formed at pH 5.5 showed the lowest gel strength (Hatta *et al.*, 1986). The influence of pH is related to its action on the net charge of proteins and to the reactivity of the sulfhydryl groups at high pH values (Croguennec *et al.*, 2002). Therefore natural pH increase in albumen during the storage of eggs increases the gel elasticity and gel rigidity (Hammershøj *et al.*, 2002). Woodward and Cotterill (1986) highlighted the influence of pH and salt concentration on the gelling of egg white ovalbumin. The authors detected a maximum of gel hardness at pH of 9.0. An addition of sodium chloride to a total value of 80 mM increased the gel hardness. These findings are in good correlation to the results of Holt *et al.* (1984), which have shown that the firmest gel is obtained for NaCl concentrations in the 0.05 to 0.1 M range. Modifications in the network formation and in the rheological properties of gels are due to the shielding of protein negative charges by sodium (Mine, 1995). It can be concluded that the balance between hydrophobic interactions and electrostatic repulsive forces influence gel turbidity and hardness.

To summarize the effects of pH and salt content, Mine (1995) proposed a model for the different gelling properties of egg white ovalbumin. At pH values far away from the IEP and at low ionic strengths, the denatured proteins exist in monomers. This is only valid in combination with low

protein concentrations of 0.05%. At a higher protein concentration of 5% linear polymers were formed. With increasing ionic strength and therefore decreasing electrostatic repulsive forces, a three-dimensional gel network is formed by interpolymer hydrophobic interactions. An explanation for this phenomenon could be given by the formation of β -sheet structures in heat denatured ovalbumin. The amount of these secondary structure elements is increased by increasing protein and salt concentrations resulting in enhanced intermolecular attractive forces. Further, Mine (1995) suggested that at high ionic strength and near the IEP, turbid and soft gels or coagula were formed by heat-denatured aggregated proteins. The presence of the coagula within the gel network resulted in the formation of opaque gels. However, the maximum gel hardness could be obtained at the critical point of gel turbidity, where the interaction between linear polymers was balanced by electrostatic repulsive forces (Mine, 1995).

To form transparent egg white ovalbumin gels with high gel rigidity, Kitabatake *et al*. (1988) suggested a two-step heating process. The colloidal solution obtained by heating ovalbumin for one hour at 80 °C in the absence of salt was mixed in a second step with NaCl solution and re-heated. Within this combination, transparent gels were obtained at 10–200 mM NaCl, and translucent gels at 300–500 mM NaCl. In contrast to those gels produced by the one-step heating method at high salt concentrations gels produced by the two-step method were much harder (Kitabatake *et al*., 1988).

As already mentioned, the reactive protein conformational state described as molten globule seems to play an important role in the formation of ovalbumin gels. Najbar *et al*. (2003) detected a compact globular form of heat treated ovalbumin in the aqueous solution by atomic force microscopy, which would be consistent with the molten globule state. In this form, the protein exists in a compact globule similar to that of the native form, with its secondary structure mostly intact but with fluctuating tertiary structure. The partial unfolding, despite the existing compact form, leads to the exposure of regions of low charge density. These newly exposed surface regions lead to increased attractive forces between the monomers. This fact gives the opportunity for monomers to build up non-covalent linkages resulting in higher molecular weight globular aggregates, the globules further linking into linear structures (Najbar *et al*., 2003).

The model proposed by Mine (1995) and the molten globule hypothesis of Najbar *et al*. (2003) are confirmed by the findings of Croguennec *et al*. (2002). The authors performed scanning electron micrographs of egg white gels. The results are depicted in Fig. 7.1. The authors detected for the pH of 5.0 a coarse and open gel network constituted of random aggregates. At a pH of 7.0, 100 to 150 nm-sized particles are clustered in a “string of beads” structure to form linear aggregates. At pH 9, a uniform gel network is observed with particle sizes from 60 to 80 nm and minimal pore size due to a high degree of cross-linking (Croguennec *et al*., 2002).

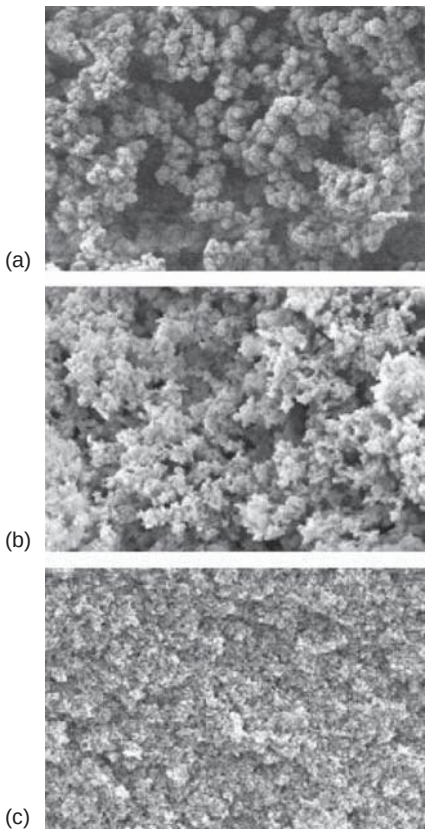


Fig. 7.1 Scanning electron micrographs of egg white gels for different pH. (a) pH 5.0; (b) pH 7.0; (c) pH 9.0. The ionic strength was kept constant at 120 mM (Croguennec *et al.*, 2002).

The type of salts also affects gel formation. It is shown by Hegg *et al.* (1979) that low concentrations of $CaCl_2$ decrease the denaturation temperature of ovalbumin. Also the quality of gels was modified on the addition of Ca^{2+} to a molar ratio of 4:1 (Ca^{2+} to ovalbumin). The authors observed a change from gel to precipitate. The observations of Croguennec *et al.* (2002) confirm these results. They detected by scanning electronic micrograph in the presence of Ca^{2+} that egg white gels were less homogeneous with particles clustered in random aggregates. The string of beads structure is decreased due to the addition of $CaCl_2$ such as those observed in the absence of cations. Ca^{2+} and Mg^{2+} ions have a similar action on egg white and ovalbumin gels, by shielding of the negative charge of ovalbumin phosphoserine residues (Mine, 1995). For the addition of Fe^{3+} ions Croguennec *et al.* (2002) analysed a uniform, fine-structured gel with a decreased

particle. It has to be mentioned that the addition of Fe^{3+} not only affects the properties of ovalbumin but also changes the properties of ovotransferrin. As discussed in Section 7.2.1 the addition of Fe^{3+} changes the denaturation temperature of ovotransferrin with an intense shift towards higher temperatures, preventing coagulation and also maintaining functionality such as foaming.

7.4.3 Foaming or whipping properties of egg white

Due to its excellent foaming properties, egg albumen is used as a functional protein ingredient in a wide range of processed foods (Damodaran *et al.*, 1998). Important criteria for good foaming properties are high foaming capacity as well as stability. Both characteristics are offered by the unique foaming properties of egg white, which are the result of the interaction between the various constituent proteins (Mine, 1995). To gain a closer insight, several studies on the foaming properties of egg white proteins have been reported in an attempt to understand the role of various constituent proteins in the expression of its surface active properties (Damodaran *et al.*, 1998; Lechevalier *et al.*, 2003, 2005a). Mine (1995) characterised three basic requirements for a protein to be a good surface active agent. Firstly proteins must have the ability to adsorb rapidly at the air–water interface during whipping or bubbling. Secondly it had to undergo a rapid conformational change and rearrangement at the interface. Finally it must provide the possibility to form a cohesive viscoelastic film via intermolecular interaction (Mine, 1995).

The foaming properties of egg white proteins are ranked in order of importance as globulins, ovalbumin, ovotransferrin, lysozyme, ovomucoid and ovomucin. It has been suggested that the different charge characteristics of the constituent proteins are responsible for the excellent foaming properties of egg (Mine, 1995; Damodaran *et al.*, 1998).

As already discussed, the basic protein lysozyme (IEP at pH 10.5) is positively charged at the natural pH of fresh egg white and can interact electrostatically with negatively charged proteins. It is believed that during foaming, both the positively charged lysozyme and other negatively charged egg white proteins migrate to the air–liquid interface. At the interface, the positively charged lysozyme interacts electrostatically with the other negatively charged proteins, and this effectively reduces electrostatic repulsive interactions in the protein film and thus stabilises the foam.

To analyse this phenomenon, Damodaran *et al.* (1998) studied the competitive adsorption of the five major egg white proteins (ovalbumin, ovotransferrin, ovoglobulins, ovomucoid and lysozyme) to the air–water interface. The relative protein concentration ratios in the bulk phase were similar to those presented in native egg white and the ionic strength was varied between a low (0.002 M) and high (0.1 M) value. The authors showed that at 0.1 M ionic strength, only ovalbumin and ovoglobulins adsorbed to

the interface. Ovotransferrin, ovomucoid, and lysozyme were essentially excluded from the interface. The surface concentration of lysozyme was measured to be essentially zero. This indicates that at 0.1 M ionic strength there were no electrostatic associations with the other egg white proteins at the interface. However, Damodaran *et al*. (1998) detected that at 0.002 M ionic strength, a significant amount of lysozyme adsorbed to the interface in combination with other egg white proteins. Therefore it is suggested that at low ionic strength lysozyme forms binary or ternary electrostatic complexes with the other proteins (Damodaran *et al*., 1998).

The findings of Damodaran *et al*. (1998) are in good correlation with the results of Pezennec *et al*. (2000). The authors analysed the surface rheological properties of ovalbumin adsorbed at the air–water interface. At a pH where the protein net charge was negative, the authors detected an increase in the final value of the shear elastic constant due to increased ionic strength.

Pezennec *et al*. (2000) suggested that interactions between adsorbed ovalbumin molecules, which form slowly in the adsorbed layer upon conformational rearrangements, impart rigidity to the interface, and that these intermolecular associations were hindered at high negative protein net charge (Pezennec *et al*., 2000). In a protein mixture the negative surface charge of ovalbumin leads electrostatic interactions with the positively charged lysozyme.

In conclusion, it has to be mentioned that for industrial applications a strong focus on the surroundings and the protein ratios must be maintained to realise constant technological functionality and product quality.

To gain a closer insight Lechevalier *et al*. (2005a) analysed the structural modifications in egg white proteins due to the adsorption at the air–water surface. They detected a synergy of denaturation if simultaneous ovalbumin, ovotransferrin and lysozyme are present in the bulk phase during foaming. In an earlier study, Lechevalier *et al*. (2003) found that lysozyme was not damaged in single-protein systems. However, in the mixture it was completely unfolded in the monomeric soluble form or involved in covalent aggregates. This phenomenon might prove the formation of intermolecular sulfhydryl–disulfide exchange reactions between ovalbumin and both ovotransferrin and lysozyme at the air–water interface (Lechevalier *et al*., 2005a).

This hypothesis was confirmed by the studies of Floch-Fouéré *et al*. (2009) who characterised the interfacial and foaming properties of different mixtures of ovalbumin and lysozyme at the air–water interface. They have shown that solutions of either ovalbumin or lysozyme exhibit different interfacial behaviour. On the one hand, ovalbumin did not form multilayers, even at high concentration. On the other hand, lysozyme formed adsorbed interfacial films that are much thicker than protein monolayers. However, the surface pressure was definitely smaller for lysozyme than that for ovalbumin.

The foaming properties of the mixtures are, however, always close to those of the pure ovalbumin solution. The authors concluded that

ovalbumin is much more surface active than lysozyme (Floch-Fouéré *et al*., 2009). It has to be considered that the used ionic strength at 0.04 M was relatively high and might prevent the lysozyme ovalbumin interactions. However, Floch-Fouéré *et al*. (2010) showed that there was a specific, stratified organisation of ovalbumin and lysozyme inside the interfacial film with an ovalbumin monolayer in direct contact with the air–water interface, which controls surface pressure, and underlying multilayers of lysozyme.

From the preceding discussion it can be concluded that egg white proteins offer a wide range of possible foaming applications in innovative food concepts. In particular, the usage of egg white proteins in a single pure form as well as in a specific mixture in combination with the control of pH and ionic strength enables promising fields of utilisation. A hot topic in the field of functional food is additives in the form of microcapsules. In this context egg white proteins might be a promising tool for the creation of adsorbed multilayer films in the range of foams and emulsion technology (Humblet-Hua *et al*., 2010).

In contrast to the results discussed in the previous section, in the food industry complex protein systems are mostly used to produce foamed products. In most cases, egg white is therefore used in combination with various proteins from different sources, e.g. caseins and whey proteins. Synergistic protein–protein interactions achieved by foaming of such protein mixtures from different sources may improve the foamed product structure. Kuropatwa *et al*. (2009) studied the interactions between whey and egg white proteins as assessed by the foamability of their mixtures. β-lactoglobulin,
the major whey protein, contains two intramolecular disulfide linkages and one free sulfhydryl group (Kuropatwa *et al*., 2009). Meanwhile, ovalbumin contains four free sulfhydryl groups and one disulfide bond (Stadelmann and Cotterill, 1995). When their functional groups are exposed, these proteins possess the potential to interact with each other by sulfhydryl/disulfide reaction. Kuropatwa *et al*. (2009) showed that the egg white proteins form foams with higher capacity and stability at pH near the IEP of ovalbumin (pH 4.5). In contrast, whey proteins show better foam properties at neutral and alkaline pH values. However, Kuropatwa *et al*. (2009) found that a synergy between whey and egg white proteins enhanced the foam capacity and stability occurring at neutral and alkaline pH when the proteins were foamed in a mixture. The synergistic effects indicating intermolecular interactions between egg white protein and whey protein occured in the bulk solution as well as after the unfolding of the proteins at the air–water interface (Kuropatwa *et al*., 2009).

Beside the protein–protein interactions in food products often protein–carbohydrate interactions alter the foaming ability of egg white proteins. Yang and Foegeding (2010) tested the effect of sucrose on egg white protein foams. The authors showed that sucrose modified the bulk phase viscosity and therefore improved the stability of wet foams. In addition to the increase of viscosity, interfacial properties of proteins could also be altered by

sucrose. Berry *et al*. (2009) suggested that the enhancing effect of sucrose (12.8% w/v) on the interfacial elasticity of egg white protein (10% w/v) contributed to increased foam stability.

7.4.4 Heat treatment of dried egg white: impact on protein functionality

The first aim of heating dried egg white powder was to reduce microbial contamination. Therefore dried egg white was generally heat-treated at 55–65 °C for several days to reduce microbial numbers from the product, according to the given legislation on hygienic safety of egg products (Mine, 1995; Hammershøj *et al*., 2006a). In addition to the effect on microbial safety, focus was set on the influence of a dry heat treatment on egg white protein functionality. At first Kato *et al*. (1989) presented promising studies of how heating egg white proteins in a dry state affected their properties. The authors found a significant improvement in foaming and gelling properties of dried egg white. Further on Kato *et al*. (1989) showed that the gel strength and foam stability of egg white proteins were increased almost fourfold due to dry heat treatment at 80 °C for 10 days.

Matsudomi *et al*. (2001) reported that dry heating of ovalbumin at 80 °C improved gel properties, including gel strengthening and transparency upon the subsequent heating for gelation. The authors suggested that the partially unfolded ovalbumin produced by dry heating forms specific soluble aggregates resulting in the formation of an ordered gel matrix (Matsudomi *et al*., 2001). As a result Lechevalier *et al*. (2007) suggested that a heat treatment of egg white powder at 75–80 °C for 10–15 days could be used in industry to offset functional property losses resulting from the spray-drying process.

Mine (1996) studied the effect of pH during dry heating on the functional properties of egg white proteins. Both gel strength and elasticity were increased in the high pH region between 8.5 and 9.5 for a short storage period of maximum 5 days. The pH of the egg white was controlled in the liquid state before spray drying. Mine (1996) reported that the polymerisation of the proteins was enhanced by alkaline pH during dry heating through sulfhydryl–disulfide interchange reactions. Alkaline dry heating resulted in high molecular weight polymer of partially unfolded egg white proteins without any loss of solubility. In contrast these aggregates were responsible for the formation of low molecular weight and narrow molecular distribution of the aggregates found during the subsequent heat for gelation. Therefore it is suggested by Mine (1996) that heating of dried egg white proteins in the dry state at pH under 9.5 is an effective method to create firm and elastic gels.

Besides the effects of the pH, Kato *et al*. (1990) and van der Plancken *et al*. (2007) pointed out that the extent of modifications induced by dry heating was related to the moisture content of the egg white powder. Kato *et al*. (1990) have demonstrated that a seven-day dry-heating treatment at 80 °C and 7.5% moisture content was responsible for the best foaming

properties. Such a treatment induced only mild structural modifications and the formation of soluble aggregates. These results suggest that controlled dry heating is an effective tool for the improvement of functional properties of egg white proteins without an associated loss of solubility. Further on, recent studies by van der Plancken *et al*. (2007) offered a closer insight in the role of the moisture content during dry heating of egg white. At 80 °C heating temperature in combination with higher moisture contents above 6.8%, the degree of protein unfolding is more pronounced. This phenomenon leads to an increased exposure of hidden SH groups and hydrophobic residues resulting in a loss of protein solubility. Thus, dry-heating at relatively low moisture content (below 6.8%) is necessary to create an egg white powder with high solubility and improved foaming properties. However, van der Plancken *et al*. (2007) reported that besides these insoluble protein aggregates, soluble, strongly stabilised small aggregates are formed, especially at higher moisture content. The oxidation of exposed sulfhydryl groups to form disulfide bonds is partly responsible for the formation of these aggregates (van der Plancken *et al*., 2007). These findings were in good correlation with the results of Mine (1996) already discussed. One possible explanation for the increased functional properties might be given by a number of studies in the field of foam stabilisation by nanoparticles or protein aggregates. It is demonstrated that small particles could have astonishing bubble-stabilising properties if they adsorb at the air–water interface. Above a certain diameter at around 10 nm, such nanoparticles can adsorb quasi irreversibly (Dickinson *et al*., 2004).

Desfougères *et al*. (2008) showed that dry-heated hen egg white lysozyme simultaneously exhibited enhanced foaming properties and aggregation capacity. The authors envisaged that heat-treated lysozyme may self-associate at the air–water interface, stabilising air bubbles. Based on their results Desfougères *et al*. (2008) hypothesised that dry-heated lysozyme with its modified structure is able to aggregate at the surface of the bubbles to form a strong cohesive film that could be responsible for the remarkable increase in foam stability. The major interactions involved in this interfacial aggregation phenomenon were hydrophobic interactions.

In contrast to the nano-particle hypothesis, the results of Desfougères *et al*. (2008) confirm the assumption of the molten globule state of dry heat-treated egg white proteins. The molten intermediate structure may be formed by controlled heating in the dry state. Therefore heat processing of egg white proteins represents a promising tool to transfer proteins in a reactive status resulting in increased functional properties.

7.5 Conclusion: egg white

Dry heating at relatively low moisture contents is necessary to create an egg white powder with high solubility and improved foaming properties.

To overcome the negative effects of spray drying and egg white processing, the application of a heat treatment of dried egg white powder seems to be a useful tool. It was demonstrated that lengthy dry-heating treatment at low moisture content was responsible for the best foaming properties. Such a treatment induced only mild structural modifications and the formation of soluble aggregates. These results suggest that controlled dry heating is an effective tool for the improvement of functional properties of egg white proteins without an associated loss of solubility. The molten intermediate structure may be formed by controlled heating in the dry state. Therefore heat processing of egg white proteins represents a promising tool to transfer proteins in a reactive status resulting in increased functional properties.

The type and concentration of salt as well as the pH determine the gelling properties of egg white. Ovalbumin is known to have a predominant role in the formation of egg white gels. The importance of pH and ionic strength on the turbidity and hardness of heat-induced ovalbumin gels was demonstrated. Depending on the environmental conditions in the medium, a transparent solution, transparent gel, turbid gel or turbid solution could be obtained due to a heating step. The gel hardness was maximal under conditions that lead to a transparent or slightly turbid gel. The effect of salt is more pronounced than the influence of pH. It was shown that the firmest gel is obtained at low NaCl concentrations. Modifications in the network formation and in the rheological properties of gels are due to the shielding of protein negative charges by sodium. It can be concluded that the balance between hydrophobic interactions and electrostatic repulsive forces influences gel turbidity and hardness. The type of salts also affects gel formation. It was shown that low concentrations of $CaCl_2$ decrease the denaturation temperature of ovalbumin. A change from gel to precipitate was observed due to the addition of Ca^{2+} ions. The influence of the amount and type of salts on the gelling properties of ovalbumin has to be kept in mind by the usage of egg white in food products.

In a protein mixture the negative surface charge of ovalbumin leads to electrostatic interactions with the positively charged lysozyme. The foaming properties of egg white proteins are ranked in order of importance as globulins, ovalbumin, ovotransferrin, lysozyme, ovomucoid and ovomucin. It has been suggested that the different charge characteristics of the constituent proteins are responsible for the excellent foaming properties of egg white. During foaming, both the positively charged lysozyme and other negatively charged egg white proteins migrate to the air–liquid interface. At the interface, the positively charged lysozyme interacts electrostatically with the other negatively charged proteins, and this effectively reduces electrostatic repulsive interactions in the protein film and thus stabilises the foam. Interactions between adsorbed ovalbumin molecules, which form slowly in the adsorbed layer upon conformational rearrangements, impart rigidity to the interface, and these intermolecular associations were hindered at high negative protein net charge. In conclusion it has to be

mentioned that for industrial applications a strong focus on the surroundings and protein ratios must be maintained to realise a constant technological functionality and product quality. Egg white proteins offer a wide range of possible foaming applications in innovative food concepts. In particular, the usage of egg white proteins in a single pure form as well as in a specific mixture in combination with the control of pH and ionic strength enables promising fields of utilisation. A hot topic in the field of functional food is additives in the form of microcapsules. In this context egg white proteins might be a promising tool for the creation of adsorbed multilayer films in the range of foams and emulsion technology.

7.6 Egg yolk: chemical composition and structure

The total dry matter of freshly laid egg yolk is about 52% ± 0.2%, and increases slightly as the laying hen gets older: an average 2% increase has been observed. The largest variation of egg yolk's dry matter takes place during storage of the eggs in their shell, because of the transfer of water from the white into the yolk. This phenomenon is due to the osmotic gradient existing in freshly layed eggs between the white (250 mOsm) and the yolk (320 mOsm) (Guilmineau, 2008). As the pH of the egg white increases during storage, the physical properties of the egg yolk membrane are modified and it allows a slow diffusion of water and small molecules from the white into the yolk (Stadelmann and Cotterill, 1986).

The solids level in commercial liquid egg yolk produced by egg-breaking machines deviates from those produced by manual separation. It is affected by many things such as the equipment, age of the egg, size of egg, etc. Usually the solids level is in the 46–49% range, depending on the amount of egg white adhering to the yolk. However, most commercial liquid egg yolk is standardised by the addition of egg white to a total dry matter concentration of 42% ± 1% (without addition of non-egg ingredients). This standardisation step is due to a transitional change in the apparent viscosity of egg yolk at a certain dry matter concentration (Stadelmann and Cotterill, 1986). The physical basics and the resulting technical aspects will be discussed later on.

Table 7.3 gives the general average composition of pure fresh egg yolk. Its dry matter is essentially composed of lipids and proteins in a ratio of 2 to 1, respectively. All lipids in egg yolk are associated in lipoprotein complexes. Lipids are composed of 62% triglyceride, 33% phospholipids and a little less than 5% cholesterol. Carotenoids, which are responsible for the yellow/orange colour of the yolk, represent less than 1% of total lipids.

7.6.1 Macrostructure of egg yolk

Egg yolk is an emulsion of lipid droplets dispersed in an aqueous protein solution. These droplets are of three types called spheres, granules and profiles (LDL).

Table 7.3 Detailed average composition of fresh egg yolk

Component	Subgroup	Main molecules	Content (% w/w)
Water			48
Lipids (34%)	Triglycerides	FA (C16:0), PUFA (C18:2), MUFA (C18:1)	22.6
	Phospholipids (9.6%)	Phosphatidylcholine (= lecithin)	7.0
		Phosphatidylethanolamine	1.4
		Sphingomyelin	0.6
		Lysophosphatidylcholine	0.2
		Lysophosphatidylethanolamine	0.2
		Plasmogen	0.1
		Inositol phospholipid	0.01
	Sterols	Mainly cholesterol	1.8
Proteins (16%)		Phosvitin	1.8
		Livetins (α -, β -, ɣ -)	5.0
		Lipovitellin (α -, β -) = HDL-apo	5.8
		Lipovitellinin = LDL-apo	3.5
Carbohydrates		Free glucose	0.2
Vitamins		Vit. A, D, E, K, B1, B2, B6, B13 ...	0.8
Minerals		P, Ca, Na, K, Cl, S, Mg, Fe	1.0

FA: fatty acid; PUFA: poly unsaturated fatty acid; MUFA: mono unsaturated fatty acid. Based on data from: Burley and Vadehra (1989); Acker and Ternes (1994); Guilmineau (2008).

Spheres have a diameter of 4 to 150 µm and are a minor constituent, representing only about 1% of egg yolk's dry matter. They appear as droplet aggregates in electronic microscopy, and would be made of lipoproteins (Bellairs, 1961). Yang (1987) has shown that a simple manual agitation is enough to dissociate these structures, so they are only present in the unbro- ken egg.

Granules have a spherical and more or less flattened shape, with an average diameter around 0.8 to 2 µm (Bellairs, 1961; Burley and Vadehra,
1979; Chang *et al*., 1977), but granula particles can be found with a diameter up to 10 µm.The density ranges between 1.089 g/ml and 1.210 g/ml.

Profiles have a diameter of about 12 to 60 nm (Martin *et al*., 1964), and have been shown to be low density lipoproteins (LDL) (Chang *et al*., 1977). As reported by Huopalahti *et al*. (2007), there are also LDL particles observable with an average diameter of 200 nm. It is possible that these structures are very low density lipoproteins as observed by Martin *et al*. (1964) or merged LDL as noted for human plasma LDL (Ala-Korpela *et al*. 1998). These findings are in good correlation to our own results. In Fig. 7.2 the particle diameter distribution of native egg yolk measured by dynamic laser light deflection is depicted. It can be seen that the LDL particles show a heterogeneous size distribution between 20 nm and 800 nm

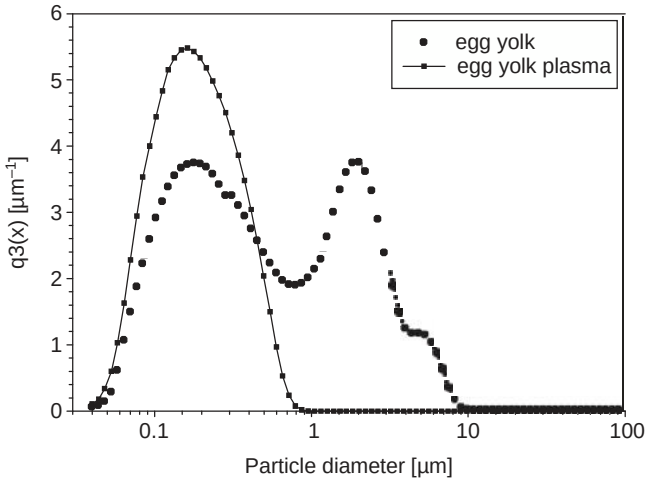


Fig. 7.2 Volume based particle size distribution $q_3(x)$ for egg yolk and the egg yolk plasma fraction prepared according to the method of McBee and Cotterill (1979). The particle size distribution is measured by dynamic light scattering in an isotonic sodium chloride solution (0.17 M NaCl, pH 6.4).

with an average diameter of 200 nm. The density of the LDL particles varies between 0.950 g/ml and 1.019 g/ml depending on the protein content.

The orange translucent liquid plasma contains the LDL and soluble proteins, which in total represents 75 to 81% of the egg yolk's dry matter. Looking at the emulsifier molecules, plasma contains 52 to 58% of all proteins and 85% of phospholipids of egg yolk. Granules contain the remaining 19 to 25% of the dry matter, which represents 42 to 48% of the proteins and 15% of phospholipids (Burley and Cook, 1961; Causeret *et al.*, 1991; Anton and Gandemer, 1997). Granules are more protein-rich than plasma and egg yolk. The lipid/protein ratio is about 0.5 for granules, whereas it is 1.9 for the whole egg yolk, and 3 to 3.3 in plasma (Dyer-Hurdon and Nnanna, 1993; Anton and Gandemer, 1997). Figure 7.3 gives an overview of the composition and structural characteristics of the granula and plasma fraction.

Granules also contain most of the di- and tri-valent cations, whereas plasma contains most monovalent cations. Over 98% of the iron, 81% of the calcium, and 71% of the magnesium are in the granules (Causeret *et al.*, 1991), whereas over 90% of the potassium and 92% of the sodium are found in the plasma (Bäckermann, 2007).

7.6.2 Constituents and microstructure of plasma

The dry matter of plasma consists essentially of 85% LDL and 15% soluble proteins known as livetins. LDL are major constituents of egg yolk: they

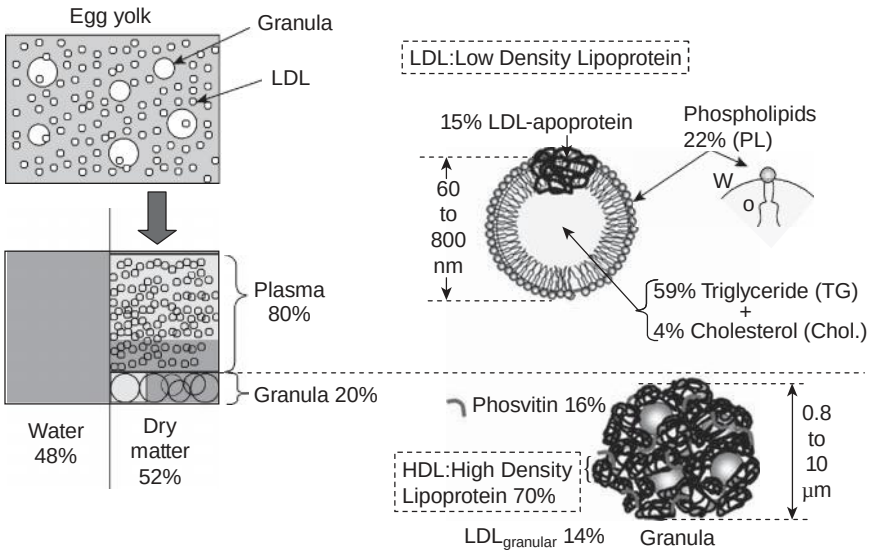


Fig. 7.3 Graphical overview of the composition and structural characteristics of the granula and plasma fraction.

represent 66% of its dry matter and contain 22% of its proteins. They are made of 83–89% lipids and 11–17% proteins. The lipids of LDL contain 74% of neutral lipids (i.e. triglyceride and cholesterol) and 26% phospholipids (Martin *et al*., 1964; Huopalahti *et al*., 2007). LDL have a typical lipoprotein structure (see Fig. 7.3), comprising a core of neutral lipids (triglycerides and cholesterol esters) surrounded by a film of phospholipids and proteins in contact with the aqueous phase. Livetins are globular proteins which are not bound to lipids and are soluble in the plasma phase of egg yolk. They represent about 11% of the total dry matter (Kiosseoglou, 2004) and about 30% of the proteins of egg yolk (Causeret *et al*., 1991). The isoelectric point of livetins ranges between 4.3 and 5.5 (Ternes, 1989).

7.6.3 Constituents and microstructure of granules

Three main components have been isolated from egg yolk granules:

Lipovitellins (also called HDL or high density lipoproteins)	70%
Phovitin	16%
Granular LDL	12%

It should be noticed that lipovitellins have been referred to as lipoproteins, although their structure is different from that of lipoproteins. They do not have a core of neutral lipids surrounded by a layer of phospholipids and apo-proteins, as is the case for LDL. Instead, they are proteins on

which lipids, mainly phospholipids, are bound as ligants by hydrophobic interactions, mainly in the N-terminal region (Banaszak *et al.*, 1982). In order to be consistent with published work lipovitellins will also be referred to as HDL. HDL represent about 16% of the total dry matter of egg yolk and about 36% of its proteins (Causeret *et al.*, 1991). The structure of HDL is held together by five disulphide bridges and comprises a hydrophobic cavity. It was shown that most HDL lipids are bound in this hydrophobic cavity (Timmins *et al.*, 1992), and that up to 40 molecules of phospholipids, organised in mono- and bi-layers, can bind to a single HDL molecule.

Phosvitin

Phosvitin represents a little over 5% of the dry matter of egg yolk, and 11% of its proteins (Causeret *et al.*, 1992; Belhomme *et al.*, 2006). Its peculiar amino acid composition is responsible for its unique functional and nutritional properties, which have been relatively well studied. It has an isoelectric point around pH 4 (Ternes, 1989). The very high content of phosphoserine is the main characteristic of phosvitin, which contains 80% of all protein-bound phosphorus in egg yolk. Phosvitin contains only about 10% hydrophobic amino acids (Belhomme *et al.*, 2006; 2008), which are grouped in the C-terminal region of the protein. The structure of phosvitin results in peculiar functional properties. This molecule has an exceptional chelating power for cations. In egg yolk, it is the main carrier of metals: it carries 95% of Fe^{3+}, for example. This has led to various studies on phosvitin, notably looking at its antioxidant properties and interfacial properties (Castellani *et al.*, 2005; 2006).

Granular LDL

Granular LDL have the same composition as the LDL present in egg yolk plasma (Sirvente *et al.*, 2007). They have been trapped within the granule structure during the formation and precipitation of granules.

Microstructure of granules

The structure of granules is held together by phosphocalcic bridges formed between phosphoseryl residues of HDL and phosvitin (Causeret *et al.*, 1992). This was shown by observing the effect of salt addition on granule structure. The addition of monovalent cations (e.g. Na^+ from NaCl) leads to a solubilisation of granule proteins and an increased concentration of free calcium. These monovalent cations displace the bivalent Ca^{2+}, which leads to the rupture of the ionic bridge between phosphoseryl residues of granule proteins. An excess of Na^+ ions over Ca^{2+} ions is, however, required, because granule constituents, and notably phosvitin, have a very high affinity for calcium (Castellani *et al.*, 2005; 2006; Tziboula and Dalgleish, 1990). It was reported that a concentration of 0.34 M NaCl only allows partial

solubilisation of granule proteins (Chang *et al.*, 1977), and a concentration of 0.5 M NaCl allows 80% solubility of egg yolk granules (Anton and Gandemer, 1997). A complete solubilisation of granule proteins is observed at concentrations of NaCl above 0.58 M (Causeret *et al.*, 1991).

Granule dissociation can also be obtained at low ionic strength by lowering the pH to values below 3.5. When the pH gets closer to the isoelectric point of phosphoseryl residues (~pH 2), the negative charge of these groups is weakened, which decreases its affinity for cations. Granule dissociation is also obtained at a pH above 7.2 because of the increased electrostatic repulsions between proteins (Causeret *et al.*, 1992).

7.7 Manufacture of egg yolk ingredients and egg yolk separation

Due to the differences in size and density, the egg yolk main fractions granula and plasma can be separated by centrifugation according to the method described by McBee and Cotterill (1979). The egg yolk is first diluted (1 : 2, w/w) in an isotonic sodium chloride solution (0.17 M NaCl) and stirred gently for 1 h before centrifugation at 10.000 *g* for 45 min at 10 °C. The supernatant plasma is collected, and the sedimented granules are washed by resuspending them in twice their volume in 0.17 M NaCl solution. To increase the purity both the plasma and washed granule fraction are then recentrifuged using the conditions described above. In Fig. 7.2 the particle size distribution of the pure plasma fraction is depicted. The purity of the plasma fraction was tested by gradient SDS-PAGE described by Guilmineau *et al.* (2005). It becomes evident that all granula particles (size range of 0.8 to 10 µ m) are removed. Further fractionation of the egg yolk constituents can be carried out as described in Fig. 7.4. Depending on the regulatory status of each country, it is possible to add 0.01% of citrate for microbial stabilisation.

7.8 Functional properties of egg yolk

A thermal treatment of egg yolk is expected to lead to some physicochemical modifications of its heat-sensitive components, particularly protein denaturation. In order to correlate changes of the techno-functional properties of egg yolk to the physicochemical modifications resulting from thermal treatment, these should be thoroughly characterised. This characterisation is the subject of the following two sections. The first one will deal with the impact of egg yolk dry matter concentration on yolk's thermal behaviour. The second part deals with the influence of environmental conditions on the solubility of native and heated egg components.

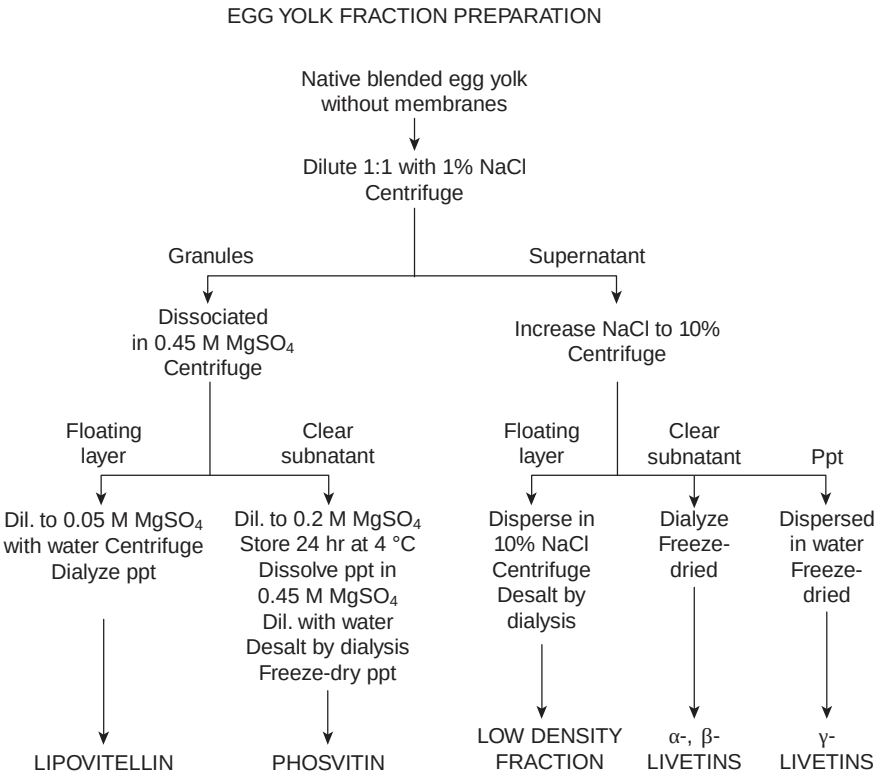


Fig. 7.4 Schematic representation of egg yolk fractionation (McBee and Cotterill, 1979).

7.8.1 Influence of egg yolk concentration on egg yolk's thermal behaviour

Ternes and Werlein (1987) used rotational viscosimetry during heating of egg yolk. They have shown that the viscosity of egg yolk increases above 65 °C and reaches a first maximum at a temperature of about 74 °C before decreasing slightly and increasing again irreversibly at temperatures above 78 °C. The authors assume that the first maximum corresponds to the denaturation of livetins, whereas the second one would be due to the aggregation of LDL and HDL apoproteins.

Le Denmat *et al.* (1999) looked at the impact of a heating at 55 to 76 °C for 150 s on the rheological properties of egg yolk, plasma and granule dispersions, all containing 24% total dry matter (0.17 M NaCl, pH 6.1). They found that the apparent viscosity of both egg yolk and plasma increased about 100-fold at temperatures above 70 °C, whereas that of granules increased about 10-fold at a temperature of 76 °C. The authors have shown that the viscosity increase in egg yolk and plasma corresponds to a decrease

of protein solubility, whereas the low solubility of granules was not affected by heating. However, Anton *et al.* (2000b) have shown that when granules are dissociated at high ionic strength (0.5 M NaCl), the solubility of HDL decreases sharply at temperatures above 72 °C, and the viscosity of the solution increases more than 100-fold.

As pointed out in a review by Kiosseoglou (2003), one should bear in mind that egg yolk is not a pure protein solution, but rather a dispersion of particles, namely LDL micelles and HDL granules. In both cases the neutral triglycerides are buried in the particle interior while the proteins dominate the particle surface, thus stabilising the system. The gelation of egg yolk can therefore be seen as the formation of an interparticle network brought about by the thermal denaturation of particle-stabilising protein molecules, somewhat similar to the heat-setting of an emulsion gel.

Anton *et al.* (2001) have shown that liquid egg yolk follows the same gelation pattern as yolk plasma, whereas granules exhibited a completely different behaviour, although they were also allowing the formation of a protein network upon cooling. The authors concluded that LDL-apoproteins control the mechanism of egg yolk gelation, notably because of their great sensitivity to heat.

The dominating role of LDL-apoproteins in egg yolk gelation was confirmed by Kiosseoglou and Paraskevopoulou (2005), who have shown that hydrophobic interactions between denatured apoproteins lead to the formation of a gel network, whereas covalent disulfide bond formation plays a complementary role. They also concluded that native granules incorporated in the network at low ionic strength act as weakening points, whereas when they are dissociated at high ionic strength, they reinforce the structure of the resulting gel.

The gelation of egg proteins is a multi-stage process, which is bound to determine the rheological and textural properties of any egg-containing food product. Most food products contain several additives such as salt and sugar. Therefore Raikos *et al.* (2007) carried out a multifactorial analysis in order to investigate the effects of pH, sugar and/or salt on the gelation process of egg yolk. The authors found out that sugar and to a greater extent salt caused an increase in the thermal transition temperature of egg proteins and subsequently raised the temperature at which they were able to form a gel. For the pH range used in their study, increasing pH also resulted in elevated gel point temperatures. Textural profiles of heat-set egg gels were all pH dependent. The gel firmness of egg yolk was particularly affected by the addition of sugar and/or salt (Raikos *et al.*, 2007).

In order to eliminate the effect of additives, Guilmineau (2008) determined the rheological behaviour of pure liquid egg yolk without the influence of salt or sugar. The flow profile of liquid egg yolk is important to know for many technical aspects like pumping, heating and storage where particles are under the effect of shear stress. Under the effect of shear, the granula and plasma particles can come in contact, interact with one another,

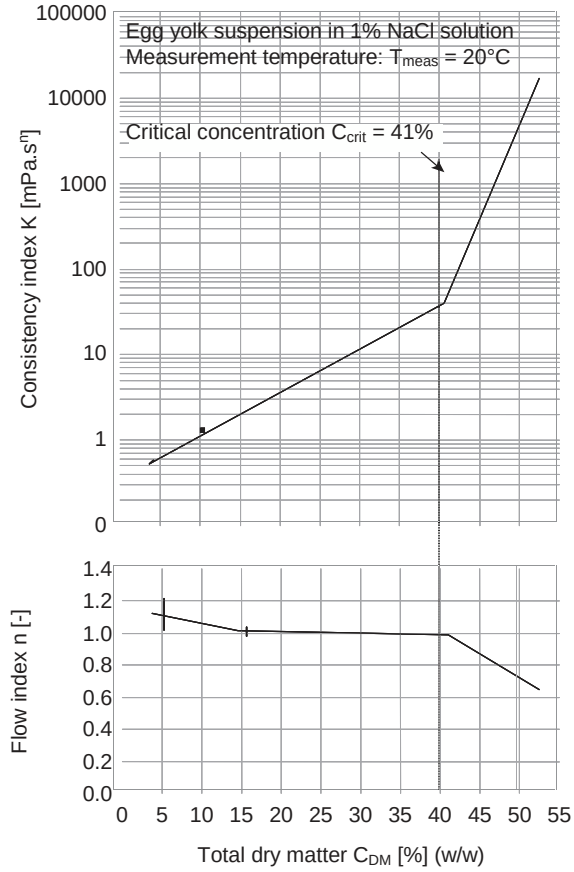


Fig. 7.5 Impact of the concentration of total dry matter on the consistency index (K) and flow index (n) of egg yolk dispersions (fresh pure egg yolk dispersed in a 1% NaCl solution) (Guilmineau, 2008).

and even undergo a certain amount of deformation. These phenomena define the flow properties of the dispersion and depend very strongly on the dry matter content, as illustrated in Fig. 7.5. The consistency index K of the dispersion increases logarithmically as the concentration of dry matter increases. This is represented by a straight line when plotting log(K) as a function of C_{DM} (Guilmineau, 2008).

Above a certain critical concentration C_{crit}, the logarithmic increase of K is greatly enhanced, which is represented by an increase of the slope of the linear relation between log(K) and C_{DM}. It was measured by Guilmineau (2008) that this critical concentration is about 41% total dry matter in the case of pure egg yolk dispersed in 1% NaCl solution.

At concentrations below C_{crit}, the increased collision frequency and energy dissipation due to friction between the particles leads to an increase in consistency with increasing dry matter concentration (Hunter, 2000). Notice that the Newtonian flow behaviour observed in this concentration range (n = 1) suggests that the continuous phase of the dispersion (i.e. livetin solution) has a Newtonian flow behaviour, and the dispersed particles are not flocculated. At concentrations above C_{crit}, the particles begin to interact with each other through a combination of hydrodynamic and colloidal interactions, which further increases the viscosity of the system (Tadros, 1994). Moreover, the flow behaviour becomes increasingly pseudoplastic as the dry matter increases above C_{crit}, which translates into a decrease in the flow index n (Fig. 7.5). This reflects the fact that the interacting particles become more ordered along the flow lines as the shear rate increases, forming "strings" or "layers" of particles which offer less resistance to the fluid flow and therefore cause a decrease in the viscosity. In order to graphically represent the change in the rheological properties of egg yolk dispersions during heating, Guilmineau (2008) defined an arbitrary six-point scale of apparent viscosity at a shear rate of 100 s^{-1} (Table 7.4). In order to render these changes more tangible for the reader, reference is made to the apparent viscosity typically encountered in common commercial products at the same shear rate.

According to the results of Guilmineau (2008), the change in consistency during heating at different temperatures is represented for the three different egg yolk concentrations in Fig. 7.6. In the dispersion containing 42.4% dry matter, the formation of a gel takes place at all tested temperatures (i.e. 63–72 °C). The heating time required for gelation decreases drastically with increasing temperature, to reach less than 5 min at 69 °C and above. When the dispersion is diluted down to 26.9% total dry matter (i.e. 50% w/w of fresh egg yolk), gelation is also observed but requires a more intensive heat treatment. At 69 °C, a gradual thickening of the dispersion is measured, which leads to gelation after 2 to 3 hours of heating. Here again, the gelation time is greatly reduced as the temperature increases, to reach less than

Table 7.4 Definition of the arbitrary viscosity levels used for the description of the gelation of egg yolk dispersions (see Fig. 7.6) (Guilmineau, 2008)

Arbitrary description	Example product	Apparent viscosity at 100 s^{-1} (mPa.s)	Symbol used
Thin liquid	Milk – sunflower oil	<100	
Thick liquid	Drinking yoghurt	100–200	
Viscous fluid	Set yoghurt	200–400	•
Thick fluid	Cream pudding	400–700	
Thick gel	Mayonnaise	700–2000	•
Pasty gel	Toothpaste	>2000	

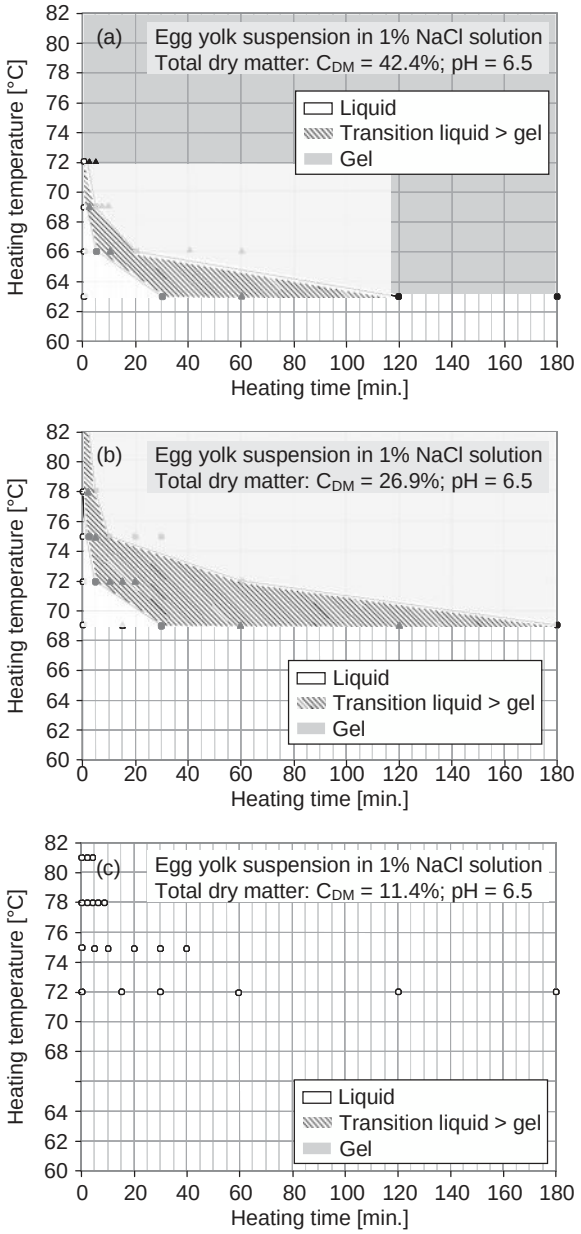


Fig. 7.6 Time/temperature conditions associated with the gelation of dispersions containing 80% (a), 50% (b) and 20% (c) fresh egg yolk in 1% NaCl solution (the legend for the symbols used in this figure is given in Table 7.4) (Guilmineau, 2008).

5 min at 78 °C and above. A further dilution down to 11.4% total solids leads to a situation whereby gelation does not take place anymore, even for the most drastic heating conditions (e.g. 81 °C for 5 min).

These results illustrate perfectly the problem of the thermal treatment of egg yolk at an industrial scale. Industrial liquid egg yolk has a dry matter content around 43–44% and contains on average about 20% egg white, which has a dry matter around 12% (Copin *et al.*, 1994). The highest dry matter content used in the work of Guilmineau (2008) is set to 42.4% and is therefore just below that of industrial egg yolk. Notice that the presence of egg white, of which proteins are reportedly even more heat-sensitive than those of egg yolk, probably renders industrial egg yolk even more prone to thermal gelation than the most concentrated dispersion produced by manual separation. In any case, the dry matter content of this product is above the critical value C_{crit} identified in Fig. 7.5. Results of Guilmineau (2008) (Fig. 7.6) suggest that industrial egg yolk would probably form a pasty coagulate for a denaturation degree as low as 5%. The pasteurisation conditions are therefore extremely limited, and even upon addition of large amounts of salt or sugar used to increase its thermal resistance, industrial egg yolk can not be heated for more than 2 min at 68 °C, or a bit longer at a lower temperature (e.g. 5–10 min at 64–65 °C is a quite standard practice). The results presented in this section demonstrate that dilution of egg yolk prior to heating allows much more severe treatments to be applied without compromising the fluidity of the product. Additionally, this result implies that a dilute dispersion can be heated much more severely than a concentrated one and still reach an equivalent degree of protein denaturation. Therefore, besides the practical advantage associated with the lower viscosity, dilution prior to heating also causes less protein denaturation for any given heat treatment.

In the light of these results, Guilmineau (2008) characterised the denaturation kinetics of egg yolk proteins in a dilute dispersion with 11.4% dry matter. This allows high degrees of denaturation, while avoiding practical problems due to the formation of an elastic gel. In Fig. 7.7 the degree of denaturation reached for any time/temperature condition is depicted. The grey area in Fig. 7.7 represents the time/temperature conditions commonly used for the pasteurisation of egg yolk at an industrial scale. Remember that industrial egg yolk has a much higher dry matter than the dispersion used to produce the results plotted in Fig. 7.7 (typically around 43–44% w/w dry matter) and is therefore much more sensitive to thermal gelation.

7.8.2 Influence of environmental conditions on egg yolk protein solubility in native and denatured state

Food products cover a wide range of pH and ionic strength that depend on compositional factors. Because of the diversity of products in which egg yolk is used as an emulsifier, it is of particular interest to understand the

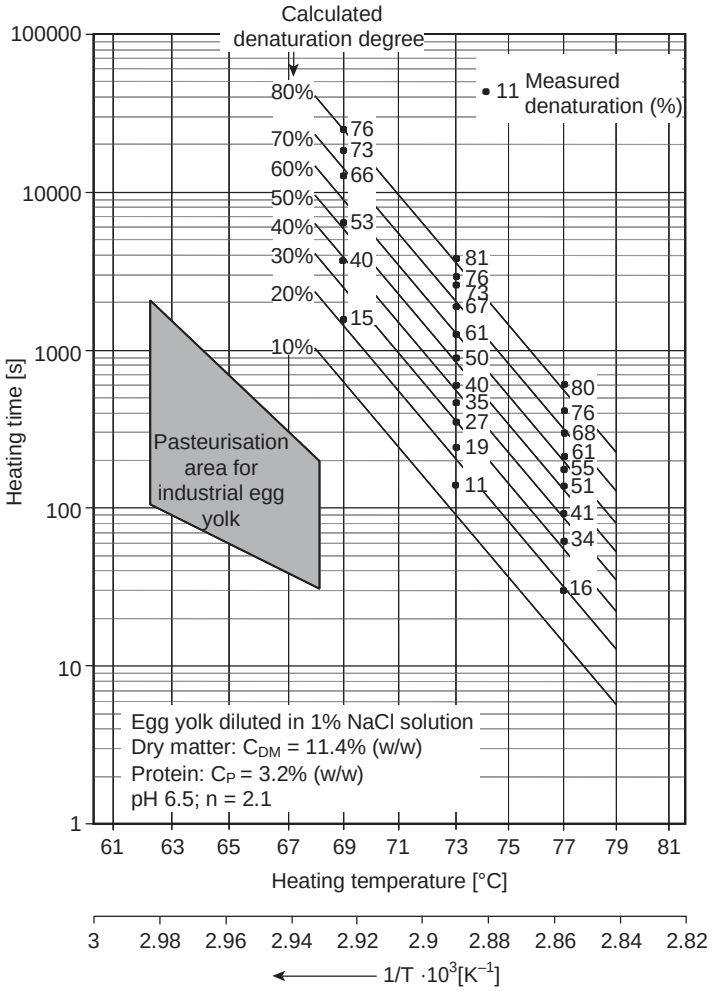


Fig. 7.7 Calculated lines of equal denaturation and measured denaturation degree of total egg yolk protein when heated in egg yolk dispersed in a 1% NaCl solution (total dry matter C_{DM} = 11.4% (w/w)) (Guilmineau, 2008).

impact of the environmental conditions on the properties of egg yolk proteins. Environmental conditions can greatly impact the structure and therefore the properties of proteins in solution. The impact of pH and ionic strength on the emulsifying properties of native and heated egg yolk will be dealt with in Section 7.8.3.

Indeed, the structural changes which modify the solubility of a protein by affecting the way it interacts with the solvent and with other proteins, also modify the way the molecule adsorbs and stabilises an o/w interface. A certain degree of unfolding under the effect of heat has a beneficial effect

on the interfacial properties of some proteins by increasing their surface hydrophobicity (Kato *et al.*, 1983; Voutsinas *et al.*, 1983). However, thermal denaturation also leads to a decrease in solubility of most proteins because of an increase in protein–protein interactions leading to aggregation. Thefore Guilmineau and Kulozik (2006a) determined the solubility of egg yolk proteins in selected environmental conditions in order to be able to correlate it to the behaviour of these proteins at the o/w interface. They tested two levels of pH. The first was pH 6.5 which is approximately the natural pH of egg yolk, and therefore represents the pH conditions in which egg yolk is placed when not included in any preparation. This is the case for industrially separated egg yolk, and when egg yolk is simply diluted with an isotonic NaCl solution. The level of pH, close to neutrality, can also be achieved in food preparations which do not include acids, such as desserts, cream toppings or most bakery preparations.

The second level was chosen by Guilmineau and Kulozik (2006a) at a pH of 4. This acid pH corresponds to the typical pH obtained in most commercially prepared salad dressing emulsions. This level of acidity is expected by the consumer for this kind of product and allows relatively good microbiological stability of the product, even when stored at room temperature. In practice, the acidity is obtained by using vinegar and/or lemon juice, which contain acetic or citric acid respectively. Guilmineau and Kulozik (2006a) decided to use an acetate buffer at pH 4 which contains acetic acid, and therefore models the use of vinegar in commercial products.

The ionic strength has a particularly relevant role when studying egg yolk proteins because of its microstructure. The authors have shown that close to complete granule solubilisation already takes place at concentrations of NaCl above 0.3 M. Therefore they decided to study the impact of granule dissociation by setting the ionic strength at two distinct levels. A low level is set at 0.15 M NaCl which corresponds to the natural ionic strength of fresh egg yolk, at which the granules are not dissociated and therefore insoluble. The high level of ionic strength is set at 0.52 M NaCl so as to obtain complete granule dissociation and therefore solubilisation. In commercial products, the ionic strength is often between these two values, and granules are therefore partly solubilised (Anton and Gandemer, 1997).

Figure 7.8 summarises the results of Guilmineau and Kulozik (2006a) by showing the impact of pH and ionic strength on the solubility of native and heated egg yolk. A dilute egg yolk dispersion in 1% NaCl solution (total dry matter C_{DM} = 11.4%) was heated for 12 min at 74°C, leading to a denaturation degree of about 57%. In native egg yolk, at a pH of 6.5, the solubility of egg yolk protein is maximal at high ionic strength: total protein solubility is close to 100%. Le Denmat *et al.* (2000) have shown that egg yolk plasma proteins retain a very high solubility (i.e. above 95%) between pH 3 and 7, and at ionic strength between 0.15 M and 0.55 M. They showed that variations of protein solubility in native egg yolk were due to variations in granule protein solubility. It was observed that the granule protein

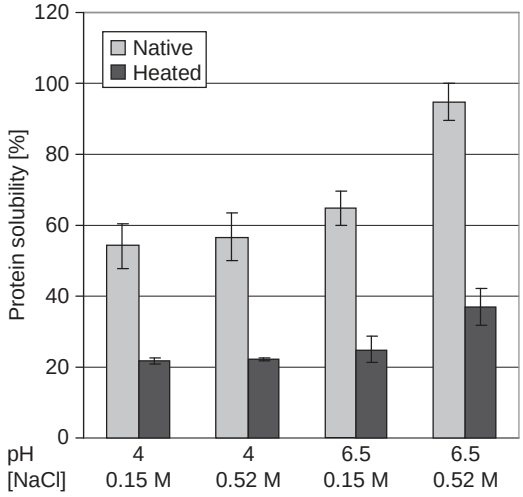


Fig. 7.8 Impact of environmental conditions on the solubility of native and heated egg yolk proteins (heating for 12 min at 74 °C) (Guilmineau, 2008).

solubility is very low (i.e. about 7%) at pH 3 whatever the NaCl concentration. Granule proteins were partially soluble at pH 7.0 and 0.15 M NaCl (i.e. about 30%) and complete solubilisation was achieved at pH 7.0 and 0.55 M NaCl. The results of Guilmineau and Kulozik (2006a) presented in Fig 7.8 confirm these observations. The solubility drop observed at pH 6.5, when the ionic strength is decreased to 0.15 M, illustrates the insolubility of granule proteins in these conditions. At a pH of 4.0, the total protein solubility drops to an even lower level, which shows the extremely low solubility of granule proteins at this pH, even when the ionic strength is as high as 0.52 M NaCl. The solubility of egg yolk proteins drops even further when the egg yolk has been partially denatured by a heat treatment at 74 °C for 12 min. However, given the extremely low solubility of granule proteins at pH 4.0, it is very probable that the remaining soluble proteins after heating (about 20% of total proteins) are plasma proteins. The fact that there are about twice as many soluble proteins at pH 6.5 and 0.52 M NaCl than at a pH of 4 suggests that some of the granule proteins are not aggregated after thermal treatment, and therefore are still native.

7.8.3 Impact of thermal treatment on the emulsifying properties of egg yolk solutions

This section deals with understanding the impact of thermal denaturation of egg yolk on its functionality in food emulsions. To give a differentiated overview two systems with different dispersed phase volume fractions are discussed. The first one is chosen with a dispersed phase volume fraction of

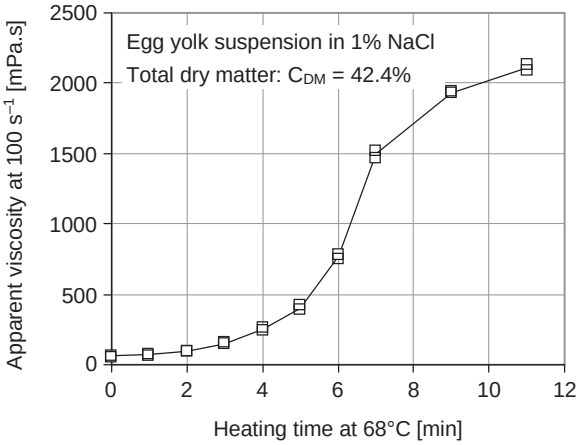


Fig. 7.9 Impact of the heating time at 68 °C on the apparent viscosity of an egg yolk dispersion (heating in a scraped-surface heat exchanger) (Guilmineau, 2008).

0.8, which is representative of typical mayonnaise. The second deals with emulsions with a oil volume fraction of 0.3 and is therefore representative of salad dressings and sauces. The approach taken in this section allows a wide range of behaviours met in commercial applications of egg yolk stabilised food emulsions to be covered.

Studies on a mayonnaise

For the manufacture of heat-treated egg yolk, Guilmineau and Kulozik (2007) prepared an egg yolk dispersion containing 80% (w/w) fresh egg yolk and 20% (w/w) of an aqueous 1% NaCl solution. The solution was heated in a scraped surface heat exchanger to obtain a homogeneous temperature distribution. The apparent viscosity of egg yolk suspension increases during heating, as can be seen in Fig. 7.9. The viscosity increase, which is relatively slow during the first 4 min at 68 °C, accelerates greatly between 4 and 8 min heating, before it starts to slow down again. As highlighted by Kiosseoglou (2003), egg yolk gelation implies a destabilisation of lipoproteins resulting from the unfolding of the apoproteins leading to attractive molecular interactions and finally to inter-particle network formation. This process was shown to involve in priority the proteins of egg yolk plasma (Anton *et al.*, 2001), and the network formation was recently reported as being dominated by hydrophobic interactions, while disulfide linkages appear to play a complementary role (Kiosseoglou and Paraskevopoulou, 2005). The initial phase observed in Fig. 7.9 (0 to 4 min at 68 °C) presumably corresponds to the time necessary for the egg yolk apoproteins to unfold under the effect of heat, before they start interacting with each other, once a critical concentration of unfolded protein has been reached (after 4 min

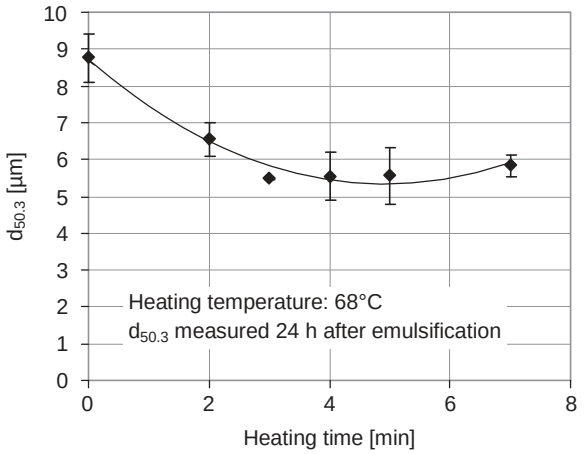


Fig. 7.10 Median oil droplet diameter in mayonnaise made with EY heated at 68 °C for different times (Guilmineau, 2008).

at 68 °C). For heating times above 7 min, the egg yolk dispersion formed a gel which was too thick to be evenly dispersed in an aqueous solution without forming large lumps (Guilmineau and Kulozik, 2007).

Figure 7.10 shows that the average diameter of oil droplets formed in mayonnaise decreases for a heating time of the egg yolk up to 4 min (Guilmineau and Kulozik, 2007). The achieved droplet size stabilises at a low value for any further heating up to 7 min. The processing parameters during the emulsification of the mayonnaise were kept constant in order to ensure a constant energy input for the disruption of oil droplets. These results suggest that the emulsifying activity of the egg yolk has been improved by the heating treatment applied prior to mayonnaise preparation. The significant decrease in oil droplet diameter in mayonnaise prepared with heated egg yolk leads to important modifications of the product's flow properties. A 4 min heating at 68 °C has been measured by Guilmineau and Kulozik (2007) to lead to a three-fold increase in the consistency index of mayonnaise, based on the Herschel-Bulkley model (Fig. 7.11). This is accompanied by increased sensitivity of the emulsion's structure to shear intensity, as indicated by the decrease in flow index from 0.45 to 0.35 (Fig. 7.11). In mayonnaise, the large contact surface area between oil droplets leads to important friction forces which oppose the free flowing of the emulsion in a shear field, hence increasing its viscosity. A decrease in oil droplet diameter leads to a greater contact surface area between droplets, and therefore to an increased viscosity (Langton *et al.*, 1999). The formation of a protein network between oil droplets under the effect of a thermal treatment in emulsions containing egg yolk, and its impact on the rheological properties of such emulsions was reported by Anton *et al.* (2001). It is,

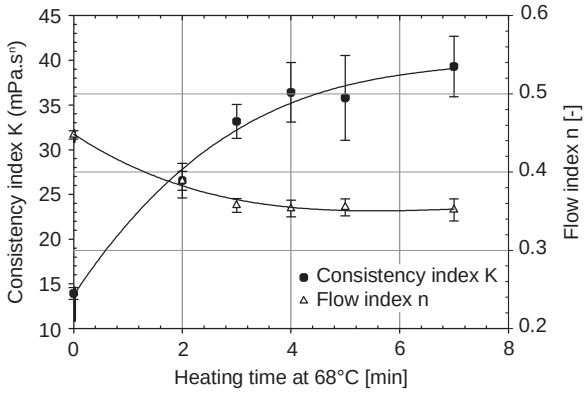


Fig. 7.11 Consistency and flow indexes in mayonnaise made with EY heated at 68 °C for different times (Guilmineau, 2008).

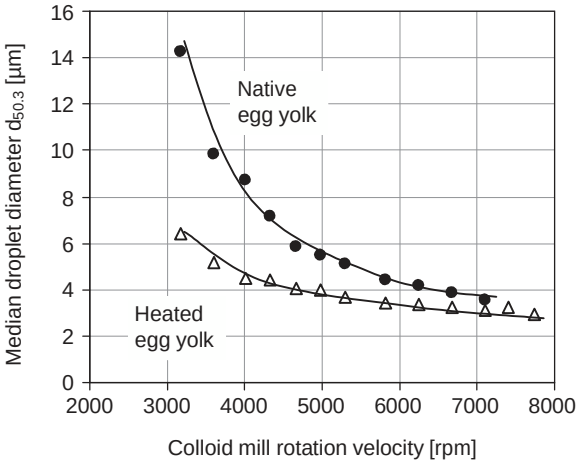


Fig. 7.12 Median oil droplet diameter in mayonnaise prepared with non-heated and heated EY (68 °C for 6 min) using different intensity of mechanical energy (Guilmineau, 2008).

however, difficult to dissociate the role played by the reduction of oil droplet size from that played by increased inter-droplet interactions, since they both lead to an increased consistency of mayonnaise. In order to look at the impact of egg yolk protein denaturation independently from the oil droplet size, Guilmineau and Kulozik (2007) looked at the influence of the energy input on mayonnaise containing either non-heated or heat-treated egg yolk (68 °C for 6 min). The corresponding results are depicted in Fig. 7.12. The difference in the achieved droplet size between non-heated and

heated egg yolk is particularly important when the rotation velocity is low, which corresponds to conditions where the energy input is the factor limiting the decrease in droplet size.

Based on the results of Guilmineau and Kulozik (2007) the following conclusion could be drawn: the mayonnaise prepared using the heated egg yolk as emulsifier was shown to have significantly different properties compared to the mayonnaise made with non-heated egg yolk. The characterisation of rheological properties of the mayonnaise suggests that the use of heated egg yolk modifies the structure of the emulsion by increasing the interactions taking place between oil droplets. The results also show that moderate thermal denaturation of egg yolk does not have any negative impact on its emulsifying properties. The formation of smaller oil droplets in mayonnaise containing heated egg yolk even suggests that the emulsifying activity of egg yolk is improved by this treatment. Furthermore it can be assumed that the amount of egg yolk and/or the energy input required to produce mayonnaise could be reduced by using partially denatured egg yolk, without affecting the final average oil droplet size of the product.

Studies on a liquid emulsion

The importance of understanding the impact of the environmental conditions on the properties of egg yolk proteins has been highlighted in Section 7.8.2, where the impact of environmental conditions on the solubility of egg yolk proteins was presented in detail. In this section the impact of the environmental conditions on the emulsifying properties of native and heated egg yolk will discussed. In contrast to the previous section, where the focus lies on the observable phenomena in emulsions containing a high oil volume fraction $\phi = 0.8$, in this section emulsion systems containing an oil phase

volume fraction ϕ of 0.3 are characterised. An oil phase volume fraction ϕ of 0.3 represents the average for the most common types of commercial salad dressings (Holcomb *et al.*, 1990). The emulsifying activity of the egg yolk is characterised by the mean oil droplet diameter in the emulsions. The lower the diameter, the better the emulsifying activity of the egg yolk. As reported by Daimer and Kulozik (2009) and Guilmineau and Kulozik (2006b), the median oil droplet diameter obtained in o/w emulsions pre- pared with non-heated and heated egg yolk is significantly influenced by the environmental conditions (Fig. 7.13). The heat treatment seems to have a rather small impact on the size of oil droplets achieved, except at a pH of 4 and a low ionic strength where heated egg yolk allows much smaller droplets than native egg yolk.

Le Denmat *et al.* (2000) found that the size of oil droplets formed in emulsions containing native egg yolk was independent from the environmental conditions. The energy used by Le Denmat *et al.* (2000) to disperse the oil by high pressure homogenisation was much higher than that used by Guilmineau and Kulozik (2006b). As could be expected, Le Denmat *et al.* (2000) obtained an average oil droplet size about 10 times smaller.

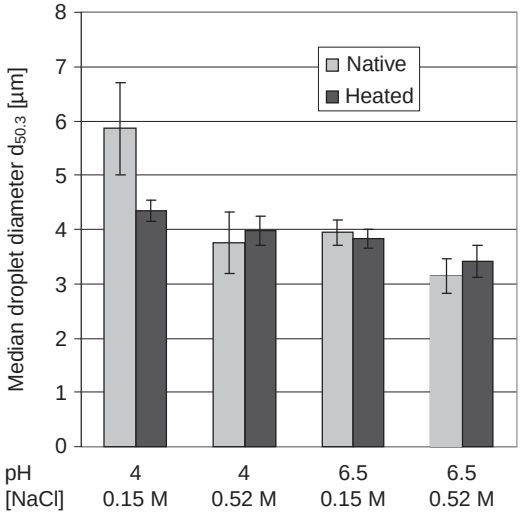


Fig. 7.13 Impact of pH and NaCl concentration on the median oil droplet diameter ($d_{50.3}$) achieved in o/w emulsions containing native (i.e. non-heated) and heated (i.e. 74 °C for 12 min) egg yolk (Guilmineau, 2008).

However, they clearly demonstrated a decreased emulsifying activity of granules at a pH of 3, which was attributed to the poor solubility of granule proteins at this pH. The authors found that this did not affect the emulsifying activity of complete egg yolk, since the same mean droplet diameter was achieved in all environmental conditions. It was concluded that the emulsifying properties of egg yolk were driven by plasma proteins. However, in the study by Guilmineau and Kulozik (2006b), where the oil was dispersed using a comparatively lower energy (i.e. single pass through one stage homogeniser at 200 bar), the authors observed an increased mean diameter in emulsions prepared at a pH of 4 and 0.15 M NaCl, indicating a decreased emulsifying activity (Fig. 7.13). This could be due to the fact that the dispersing forces generated during emulsification were insufficient to allow a shear-driven dissociation of granule proteins at this low pH and ionic strength. Anton *et al.* (2000a) showed that insoluble egg yolk granules do adsorb at the o/w interface, and suggested that fragments of granules obtained during homogenisation could also play the same role. As the intensity of disruption forces increases, the partial dissociation of granules leads to a more efficient adsorption of granule fragments at the o/w interface, and an overall better surface activity of egg yolk. Guilmineau (2008) observed that the emulsifying activity of egg yolk was high at a pH of 4 and 0.55 M NaCl (Fig. 7.13), despite the low protein solubility. This indicates that the presence of high concentrations of NaCl facilitate the shear-induced dissociation of insoluble egg yolk granules at pH 4, even when relatively low shear forces are used.

When an egg yolk dispersion is heated, part of the granule proteins are denatured and form heterogeneous aggregates together with denatured egg yolk plasma proteins. The forces bonding proteins within heat-coagulated egg yolk have been shown to be mostly hydrophobic interactions (Kiosseoglou and Paraskevopoulou, 2005). These interactions are quite weak and can probably be dissociated by the shear forces in an one stage homogenisation step at 200 bar. This hypothesis is supported by the findings of Sirvente *et al.* (2007). The authors found that the granula constituents of liquid egg yolk dispersions can adsorb at the oil–water interface as whole aggregates or as fragments disrupted during the homogenisation process. The interfacial film in emulsions prepared with heat-treated egg yolk is formed by the adsorption of a multitude of minute fragments of thermally aggregated proteins, obtained by the shear-driven disruption of much larger protein aggregates during high pressure homogenisation. These findings are supported by the fact that denatured egg yolk proteins are adsorbing at the o/w interface, even though they form insoluble protein aggregates in the continuous phase (Guilmineau, 2008). The results highlight the importance of a controlled energy input during the emulsification step when egg yolk is used.

Besides the droplet size, the interfacial protein concentration characterises the properties of an emulsion. It was shown by Guilmineau and Kulozik (2006b) that in emulsions prepared with native egg yolk the interfacial protein load is significantly higher at a pH of 6.5 than at a pH of 4 (Fig. 7.14). Additionally the ionic strength does not seem to influence the

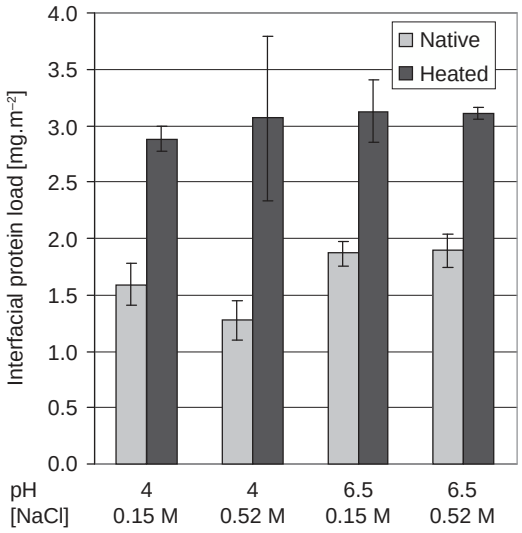


Fig. 7.14 Impact of pH and NaCl concentration on the interfacial protein concentration measured in o/w emulsions containing native (i.e. non-heated) and heated (i.e. 74 °C for 12 min) egg yolk (Guilmineau, 2008).

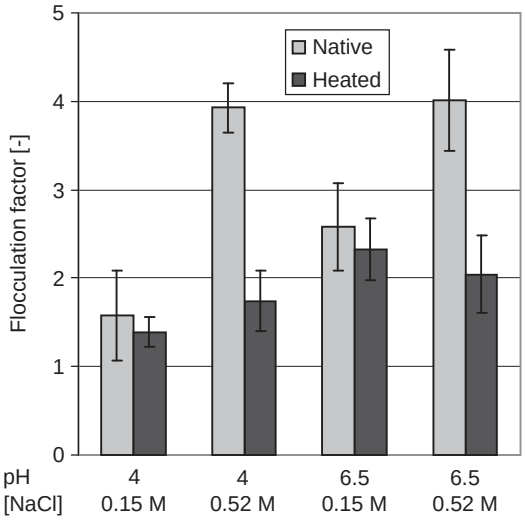


Fig. 7.15 Impact of pH and NaCl concentration on the flocculation factor measured in o/w emulsions containing native (i.e. non-heated) and heated (i.e. 74 °C for 12 min) egg yolk (Guilmineau, 2008).

interfacial protein load at pH 6.5, but at pH 4 the interfacial load is lower at an ionic strength of 0.52 M NaCl than at an ionic strength of 0.15 M NaCl. On the other hand, when heated egg yolk is used, the interfacial protein concentration is much higher than when native egg yolk is used. Moreover, there does not seem to be any negative impact of the environmental conditions on the interfacial protein load, since the differences measured between the various conditions tested are not significant (Guilmineau and Kulozik, 2006b).

Guilmineau (2008) has shown that both the environmental conditions and heat treatment had a very significant impact on the flocculation of oil droplets (Fig. 7.15). The flocculation factor of emulsions prepared with native egg yolk was always higher than that of emulsions prepared with heated egg yolk, under all environmental conditions tested (Guilmineau, 2008). With native egg yolk, the ionic strength seems to drive the flocculation, whereby a high ionic strength leads to the largest level of flocculation, regardless of the pH. When heated egg yolk is used as opposed to native, the level of flocculation in emulsions containing 0.52 M NaCl is greatly reduced, down to a level similar to that obtained at low ionic strength. The flocculation factor seems to be less dependent on the environmental conditions (Guilmineau and Kulozik, 2006b).

An important fact for the shelf life of emulsions is their stability against creaming. Therefore Guilmineau (2008) tested the initial creaming rate of

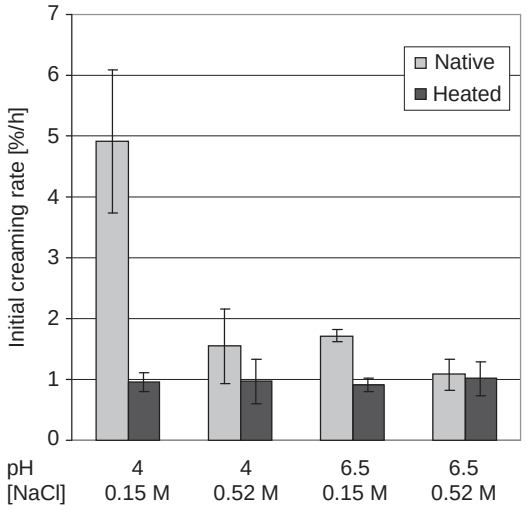


Fig. 7.16 Impact of pH and NaCl concentration on the initial creaming rate (first 6–11 hours after emulsification) measured in o/w emulsions containing native (i.e. non-heated) and heated (i.e. 74 °C for 12 min) egg yolk (Guilmineau, 2008).

emulsions 18 hours after the emulsification step. For the emulsion prepared with native egg yolk, the author measured that the creaming rate of oil droplets is much higher at pH 4 and 0.15 M NaCl than under any other environmental conditions (Fig. 7.16). However, in emulsions prepared with heated egg yolk, there is no significant impact of the environmental conditions on the creaming rate. The creaming rate is always slightly lower when heated egg yolk rather than native egg yolk is used as emulsifier.

Notice that emulsions made with native egg yolk have a tendency to flocculate more than those made with heated egg yolk, which reflects the presence of greater attraction forces between oil droplets. This can lead to oil droplets sticking together when coming in contact during creaming, and therefore forming a cream layer with a relatively open structure. This could explain why emulsions containing denatured egg yolk, which tend to be less flocculated, also tend to form a more compact cream layer than those made with native egg yolk (Guilmineau and Kulozik, 2006b).

The use of thermally denatured egg yolk as emulsifier significantly modifies the properties of the obtained liquid emulsion, as well as the impact of the environmental conditions on these properties. When heated egg yolk is used, the disruption of protein aggregates and their adsorption at the interface takes place independently from environmental conditions. It can be concluded that a partial denaturation of egg yolk proteins prior to the formation of an emulsion has a rather positive effect on the properties of the final product over a wide range of pH and salt concentrations.

7.8.4 Impact of an enzymatic treatment via PLA_2 on the properties of egg yolk proteins

Industrial egg yolk has to be pasteurised to ensure microbiological safety. If the egg yolk is not diluted, as described in the previous section, only low temperature-time combinations can be applied for pasteurisation because of egg yolk's high total dry matter, high protein content, and tendency to block heat exchangers because of heavy fouling. Nowadays, phospholipase A_2 (PLA_2) is used in the egg industry to improve the heat stability of egg yolk and enhance its functionality (Dutilh and Groger, 1981). PLA_2 cuts the acyl group in position 2 of the triglyceride and converts the phospholipids into lyso-phospholipids, which show a higher solubility in water and therefore might improve the emulsifying properties of egg yolk in o/w emulsions. Figure 7.17 gives a schematic representation of enzyme hydrolysis. Such modified egg yolk does not gel even under severe heat treatment and can therefore be pasteurised at higher temperatures. The degree of conversion of egg yolk phospholipids depends on the amount of enzyme added, the reaction temperature, the incubation time and the salt content of the egg yolk. The calcium concentration of egg yolk is sufficient to give an optimal reaction rate for phospholipase A_2, which needs Ca^{2+} ions for activity (Dutilh and Groger, 1981). As described by Daimer and Kulozik (2008), an optimum of incubation parameters was reached at a temperature versus time combination of 55 °C for 3 h, respectively. The pH of the suspensions was 6.10 ± 0.03. The egg yolk was incubated at a total dry matter of 44%, diluted in an isotonic salt solution containing 0.17 M NaCl. 0.4 µ L of enzyme/g of egg yolk suspension (lactivity of 10 000 units/mL) was added.

7.8.5 Impact of an enzymatic treatment via PLA_2 on the thermal behaviour of egg yolk proteins

Enzymatic modification via PLA_2 improves the heat stability of egg yolk (Mine, 1997). Figure 7.18 presents the denaturation degree of proteins of

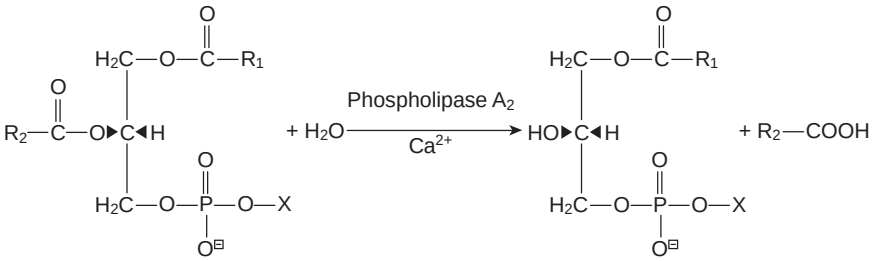


Fig. 7.17 Schematic representation of the enzymic hydrolysis of the ester bond at the C-2 position of phosphoglycerides by phospholipase A_2. Group X represents any of the naturally occurring residues, which are found in phosphoglycerides (Dutilh and Groger, 1981).

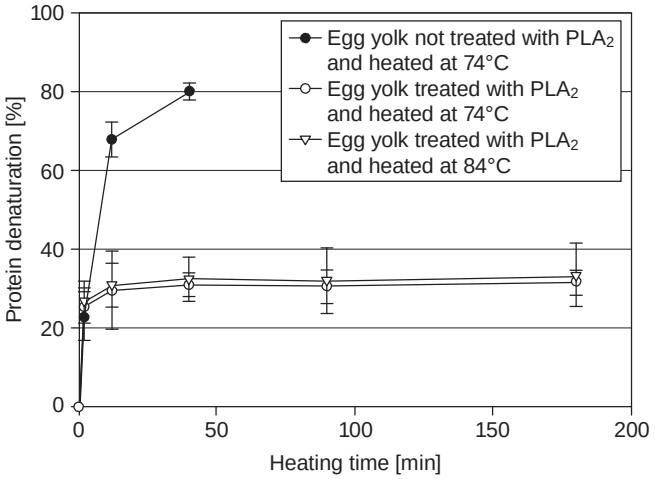


Fig. 7.18 Denaturation of proteins in whole egg yolk without enzymatic treatment, heated at 74 °C for 0–40 min, and after treatment with PLA_2, heated at 74 and 84 °C for 0–180 min (Daimer, 2007).

untreated and PLA_2-treated egg yolk (Daimer and Kulozik, 2008). The egg yolk was heated at 74 °C without prior enzyme treatment, leading to a protein denaturation of 30% in 2 min and to a further increase in denaturation up to 80% in 40 min of heating. This result is in agreement with Guilmineau and Kulozik (2006a). Natural egg yolk was not heated further, because the egg yolk suspension began to gel. PLA_2-treated egg yolk shows the same degree of protein denaturation at 74 and 84 °C. While 80% of all proteins were denatured in natural egg yolk, the PLA_2 treatment led to a maximum degree of protein denaturation of only 40%. This means that at least 60% of all egg yolk proteins are heat-stable under these conditions when the egg yolk was treated by PLA_2. Mine (1998a) suggests a heat-stable complex formed between LDL apoproteins resulting in a closer and tighter packing of the surface. The enhanced structural rigidity of the micelles surface precludes particle fusion (Hevonoja *et al.*, 2000). This observation can be transferred to the effect that no aggregates are formed during heat treatment of the egg yolk plasma. Because the LDL particles are not likely to aggregate because of their increased surface rigidity induced by lyso-PL, no insoluble aggregates can form during heat treatment and, therefore, the plasma proteins remain soluble. Because granules contain 12% LDL, the lower denaturation degree of granule fraction after enzymatic treatment can be explained by the heat stability of LDL apoproteins.

7.8.6 Impact of a PLA_2 treatment on the solubility of egg yolk proteins

As highlighted in Section 7.8.2, the solubility of egg yolk's proteins strongly depends on the environmental conditions. Additionally, an impact of

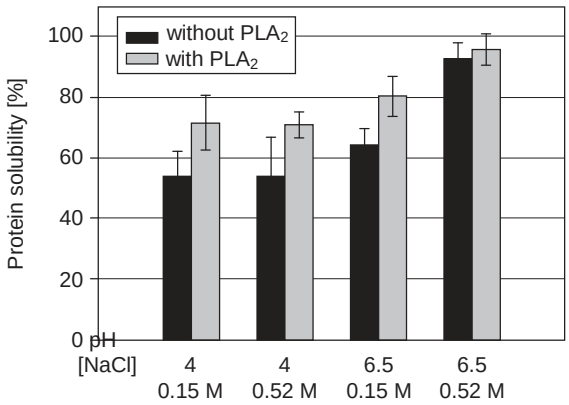


Fig. 7.19 Protein solubility at different pH and ionic strength of untreated (without PLA_2) and enzyme-treated (with PLA_2) egg yolk suspensions (Daimer, 2007).

enzymatic pre-treatment of egg yolk is observed (Daimer and Kulozik, 2008; 2009).

Figure 7.19 shows the protein solubility level of egg yolk, which is not treated or treated with PLA_2, in four different environmental conditions. At pH 6.5 and 0.15 M NaCl, egg yolk is in its natural environmental conditions. Protein solubility for non-treated egg yolk and PLA_2-treated egg yolk is 63% and 80%, respectively. When NaCl concentration is increased to 0.52 M NaCl at pH 6.5 proteins of both egg yolk types, non-treated and treated with PLA_2, are nearly completely soluble. Most emulsions containing egg yolk have a pH around 4 and therefore this pH is relevant to reflect an environment of a dressing or sauce. The lowest protein solubility is observed at pH 4 for non-treated egg yolk as well as enzyme-treated egg yolk. At this pH, granules are insoluble as described by Le Denmat *et al.* (2000). Solubility of granules is responsible for the solubility of whole egg yolk as plasma proteins are soluble in each condition used in the study by Sousa *et al.* (2007). Modified egg yolk shows higher protein solubility at all environmental conditions used. However, the solubility of enzymatically modified egg yolk still depends on pH and ionic strength. As reported by Daimer and Kulozik (2008), the higher protein solubility of modified egg yolk results from a breakdown of the highly aggregated granule structure. Granule proteins can be solubilised after modification by PLA_2, even when pH and ionic strength are low (Gorshkova *et al.*, 1996). The mechanism of granule breakdown into smaller fragments is not yet clear. It may be similar to the effect of higher salt concentrations, where sodium ions replace calcium ions and therefore disrupt phosphocalcic bridges between HDL and phosvitin. More likely, however, is that granules lose their dense aggregated structure due to structural changes within LDL micelles (12% in

granules) after enzymatic modification by PLA_2 (Hevonoja *et al.*, 2000). The results presented in Fig. 7.19 lead to the assumption that not only lysophospholipids, which result from the enzymatic reaction, should be made responsible for the effect on functionality. From the observations of Daimer and Kulozik (2008, 2009) the conclusion could be drawn that egg yolk's proteins are also indirectly affected by PLA_2, which primarily acts on phospholipids only. It would seem that the PLA_2 reaction causes structural changes of granules and allows proteins to leave the complex granular structure. Hence, higher concentrations of soluble proteins in the continuous phase are available to act as interfacially active components. This, in turn, is expected to improve the emulsifying activity of the egg yolk system. Therefore, the following sections discuss the indirect effect of PLA_2 on the emulsification process.

7.8.7 Impact of a PLA_2 treatment on the emulsifying activity of egg yolk proteins

As is known from Daimer and Kulozik (2009), enzymatic treatment of egg yolk via PLA_2 changes not only the thermosensitivity of egg yolk but also the techno-functional properties of protein compounds. Figure 7.20 presents the median oil droplet diameter obtained from o/w emulsions when untreated and modified egg yolk was used, depending on different environmental conditions. The experiments were conducted in a way comparable to the studies by Guilmineau and Kulozik (2006b) using a moderate energy input (200 bar, one-stage homogenisation) and a protein concentration of 20 mg/ml. All median oil droplet diameters achieved with modified egg yolk were smaller than with untreated egg yolk. This result reflects an increased

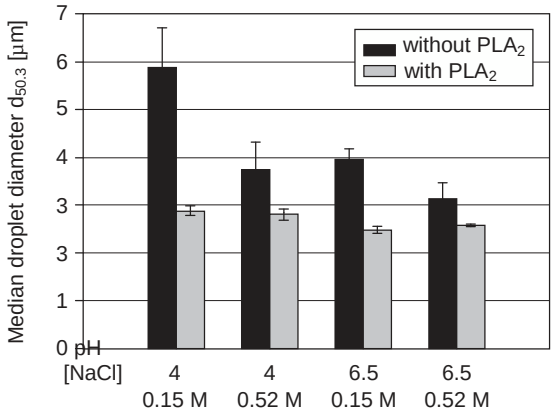


Fig. 7.20 Impact of pH and NaCl concentration on the median oil droplet diameter of oil-in-water emulsions containing untreated (without PLA_2) and enzyme-treated (with PLA_2) egg yolk (Daimer, 2007).

emulsifying activity of PLA_2-treated egg yolk. Results for egg yolk which were not treated by PLA_2 are in agreement with results of Guilmineau and Kulozik (2006b). The largest droplets are found at more acidic pH and lower ionic strength where granules are insoluble. However, a remarkable decrease in droplet diameter is observed when egg yolk was treated with PLA_2 prior to emulsification (Daimer and Kulozik, 2009). The emulsifying activity of modified egg yolk also seems to be independent from ionic strength and only slightly dependent on pH. The droplet size obtained after high-pressure homogenisation can be attributed to the ability of the emulsifier to reduce the interfacial tension but also to its capacity to prevent re-coalescence of newly formed droplets within and just after the valve of the homogeniser. The turbulence in the high-pressure homogeniser enhances collisions between newly formed droplets and therefore increases the probability of their re-coalescence. The smaller granule fragments in PLA_2-treated egg yolk can cover the interface more completely than large aggregates, e.g. granules in untreated egg yolk, which leads to a lower interfacial tension and therefore a lower droplet size is achieved. Furthermore, this result reflects that the emulsifying activity of untreated egg yolk strongly depends on the solubility and the structure of granules and highlights the role of protein monomers which have become soluble due to PLA_2 treatment as emulsifiers. As granules are disrupted anyway by the pre-treatment with PLA_2, no additional effect of the shear stress occurring in the high-pressure homogeniser was observed (Daimer and Kulozik, 2009).

To characterise the stability of an emulsion, the flocculation and the initial creaming rate are of major interest. The flocculation factor between oil droplets depending on the environmental conditions for non-enzyme-treated and enzyme-treated egg yolk is shown in Fig. 7.21. Emulsions from

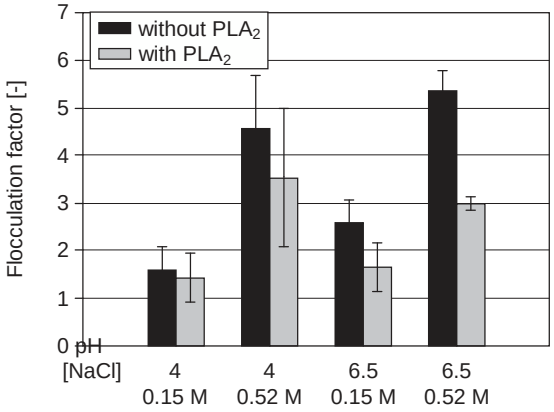


Fig. 7.21 Impact of pH and NaCl concentration on the flocculation factor of oil-in-water emulsions containing untreated (without PLA_2) and enzyme-treated (with PLA_2) egg yolk (Daimer, 2007).

both egg yolk types show higher flocculation when NaCl concentration was 0.52 M. At pH 6.5, the level of flocculation in emulsions containing enzyme-treated egg yolk is significantly lower than in emulsions containing untreated egg yolk. However, at pH 4 and 0.52 M NaCl the difference in flocculation factor is not as significant, but a similar trend can be seen. At pH 4 and 0.15 M NaCl flocculation seems to be identical for both, untreated and enzyme-treated egg yolk.

The phenomena of flocculation and creaming are closely related. Usually flocculation leads to enhanced creaming velocity because of the higher effective droplet size, i.e. droplet aggregates. Therefore the initial creaming rate of emulsions during the first 18 hours after emulsification was assessed to measure the immediate impact of the PLA_2 treatment (Daimer and Kulozik, 2009). The authors have shown that creaming rates of emulsions containing non-modified egg yolk were higher compared to creaming rates of emulsions containing enzyme-treated egg yolk (Fig. 7.22). The highest creaming rate was measured at pH 4 and 0.15 M NaCl for the emulsion without PLA_2 which is in accordance with results from Guilmineau and Kulozik (2006b). The lowest creaming rate found for emulsions without PLA_2 was at pH 6.5 and 0.52 M NaCl. Creaming rates for emulsions with PLA_2 were below 1%/h regardless of the pH and ionic strength used. It was shown that despite a higher flocculation factor in emulsions containing high salt concentrations, there is no subsequent impact on the emulsion properties, i.e. the rheological and creaming behaviour. Therefore, it is assumed that forces leading to flocculation are weak in emulsions containing enzyme-treated egg yolk. Results clearly demonstrate that PLA_2-treated egg yolk can improve emulsion properties at low pH and that this effect can be

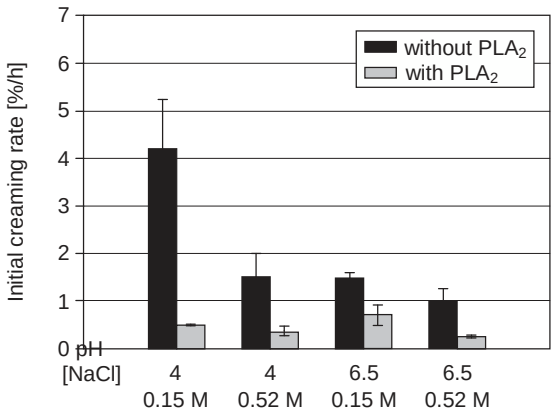


Fig. 7.22 Impact of pH and NaCl concentration on the initial creaming rate (18 h after emulsification) of oil-in-water emulsions containing untreated (without PLA_2) and enzyme-treated (with PLA_2) egg yolk (Daimer, 2007).

assigned to the higher protein solubility of modified egg yolk. In conditions where egg yolk proteins are completely soluble anyway (pH 6.5 and 0.52 M NaCl), emulsion stability is not further improved by an enzymatic pre-treatment of the egg yolk.

Comparing the results of the impact of heat treatment and enzymatic modification on the techno-functional properties of egg yolk proteins, the following conclusion can be drawn. It is possible to reach nearly the same emulsion characteristics by the use of thermal treatment of native liquid egg yolk as is reached by the application of PLA_2 enzymatic-treated egg yolk. Due to dilution of the liquid egg yolk prior to heat treatment, the degree of protein denaturation could be adjusted exactly. Therefore the quality characteristics of liquid egg yolk with increased functionality could be adjusted to product requirements. Heat treatment in combination with a dilution of natural egg yolk is supposed to be an appropriate alternative for PLA_2-treated egg yolk for producing stable emulsions.

An advantage of the use of PLA_2-treated egg yolk for the preparation of emulsions is given by Daimer (2007). The heat sensitivity of prepared emulsion could be considerably decreased due to PLA_2-modified egg yolk. This result might give the opportunity to pasteurise prepared emulsions at moderate conditions to improve the shelf life.

7.8.8 Impact of enzymatic treatment via PLD on the properties of egg yolk

In the last few years another possibility for the phospholipid modification of liquid egg yolk has come in the focus of the food industry. Phospholipase D (PLD) hydrolyses the phosphate ester group of phospholipids. If egg yolk lecithin is used as substrate, mainly choline and phosphatidic acid are formed. Additionally, PLD modification can cause a transphosphatidylation with other polar groups, e.g. alcohols, forming phospholipids with modified head groups. PLD from *Streptomycees chromofuscus* is often used to investigate the behaviour of this type of phospholipase (Buxmann *et al.*, 2010b). The enzymatic activity of PLD depends on the availability of calcium ions in the substrate solution. It is shown by Buxmann *et al.* (2010a) that a treatment of egg yolk phospholipids by PLD releasing the head group, e.g. choline in the case of phosphatidylcholine, and forming phosphatidic acid significantly influences functionality of the egg yolk. The authors observed an increase in egg yolk viscosity caused by formation of networks between protein side groups liberated through perturbation of surface structures of LDL during enzymatic treatment. Regarding application of such an egg yolk in o/w emulsions, an increase in emulsion viscosity was also observed by Buxmann *et al.* (2010a). As depicted in Fig. 7.23 the viscosity increase was most obvious in the emulsions with the highest protein content indicating that the aggregation of proteins in the aqueous phase and their interactions with oil droplet surface coverings was the main reason for the higher

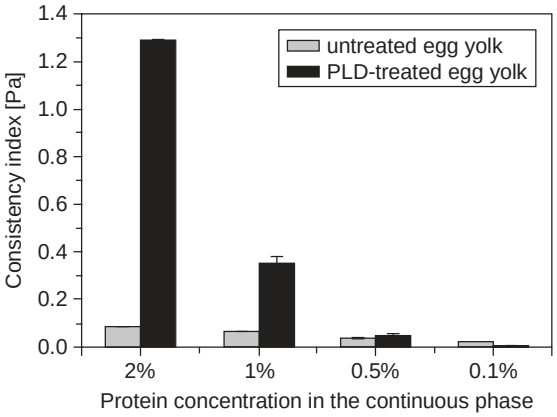


Fig. 7.23 Flow consistency index of o/w emulsions (70/30, w/w) prepared with untreated egg yolk and egg yolk after incubation with phospholipase D (PLD-treated) with different protein concentrations in the continuous phase of the emulsion (Buxmann *et al.*, 2010b).

viscosity. Furthermore, improved emulsifying activity of PLD-treated egg yolk was detected in emulsions with protein contents of 1% and lower corresponding to an egg yolk content of about 5% in the emulsion (Buxmann *et al.*, 2010a). In Fig. 7.23 the mean oil droplet diameter in o/w emulsions (70/30, w/w) prepared with untreated egg yolk and egg yolk after incubation with phospholipase D is depicted. It becomes obvious that the effect of an enzymatic treatment via PLD on the emulsifying activity is quite moderate. However it is shown by Buxmann *et al.* (2010a) that there is increased heat stability of prepared emulsions. Application of PLD-treated egg yolk resulted in a much lower increase in oil droplet diameters due to heating, indicating improved heat stability of such an emulsion. This might give the possibility to pasteurise emulsions prepared with PLD-modified egg yolk to extend their storage stability and shelf life.

7.9 Conclusion: egg yolk

Diluting egg yolk prior to thermal treatment presents some advantages regarding the denaturation of its proteins

The formation of a pasty gel upon heating results from the very high protein content of natural egg yolk. Increasing the distance between molecules by artificially diluting the egg yolk prior to heating was shown to slow down the thermal aggregation of egg yolk proteins. The formation of discrete protein aggregates in diluted egg yolk, as opposed to a continuous network, limits the increase in viscosity of the product upon heating. This renders the

product easy to handle for industrial use. Many formulated industrial products containing egg yolk also have free water in their ingredient list. For such products, it would be possible to use a dilute egg yolk solution and remove the corresponding amount of water from final formulation. This represents the scope of application of long-life egg yolk suspensions having undergone more severe heat treatments than currently available pasteurised products. Besides the convenience associated with the extended shelf life, a given protein denaturation degree could be targeted, with the objective to retain or even improve the functional properties of egg yolk.

Thermal denaturation of egg yolk leads to improved emulsifying performance in the preparation of concentrated emulsions

It is shown that using partially denatured egg yolk rather than native for the preparation of mayonnaise leads to the formation of smaller oil droplets. This is believed to be due to the increased level of colloidal interaction taking place between the denatured proteins, and leading to an increase in the viscosity of the continuous phase of the emulsion. The fact that oil droplets remain small shows that the heated egg yolk was able to quickly form a cohesive film around the droplets and prevent re-coalescence. Moreover, the discussed results prove that a partial protein denaturation of egg yolk can be used as a way to modify the textural characteristics and the stability of a mayonnaise without necessarily impacting the oil droplet size. This allows a better decoupling of the textural attributes of the product from other characteristics impacted by the oil droplet size, such as flavour release, for example.

Egg yolk allows the formation and stabilisation of liquid emulsions even at very high levels of thermal denaturation

It was shown that denatured egg yolk proteins do adsorb at the o/w interface, and form an elastic and cohesive film, able to ensure long-term stabilisation against droplet coalescence. Furthermore it could be demonstrated that the interfacial protein concentration is positively correlated to the degree of protein denaturation. It appears that the adsorption of protein aggregates at the interface leads to the formation of a denser and possibly thicker interfacial membrane. This is thought to increase the level of steric repulsions between stabilised oil droplets, thereby preventing flocculation.

The use of thermally denatured egg yolk as emulsifier significantly modifies the properties of the obtained liquid emulsion, as well as the impact of the environmental conditions on these properties

When heated egg yolk is used, the disruption of protein aggregates and their adsorption at the interface takes place independently from environmental conditions. It can be concluded that a partial denaturation of egg yolk proteins prior to the formation of an emulsion has a rather positive effect on

the properties of the final product over a wide range of pH and salt concentrations. The formation of small oil droplets during emulsification can thereby take place even in conditions which are unfavourable for the formation of emulsions with non-heated egg yolk, such as at low pH and ionic strength. Similarly, it is demonstrated that the increased interfacial protein load resulting from the use of denatured egg yolk proteins is independent from environmental conditions.

Enzymatic modification via PLA_2 and PLD result in improved heat sensitivity of prepared emulsion; however, the techno-functional benefit of a PLA_2 modification could be substituted by a moderate thermal treatment of diluted egg yolk

It is possible to reach nearly the same emulsion characteristics by the use of a thermal treatment of native liquid egg yolk as is reached by the application of PLA_2 enzymatic-treated egg yolk. Due to a dilution of the liquid egg yolk prior to heat treatment, the degree of protein denaturation could be adjusted exactly. Therefore the quality characteristics of liquid egg yolk with increased functionality could be chosen to product requirements. Heat treatment in combination with a dilution of natural egg yolk is supposed to be an appropriate alternative for PLA_2-treated egg yolk for producing stable emulsions.

The heat sensitivity of prepared emulsions could be considerably decreased due to PLA_2-modified egg yolk. This result might give the opportunity to pasteurise prepared emulsions at moderate conditions to improve shelf life.

Application of PLD-treated egg yolk resulted in a much lower increase in oil droplet diameters due to heating, indicating an improved heat stability of such an emulsion. This might give the possibility to pasteurise emulsions prepared with PLD-modified egg yolk to extend their storage stability and shelf life.

7.10 Regulatory status: egg proteins as food allergens

Specifications and standards are published by governmental regulatory agencies and by egg product producers and customers. They are the basis for the consistent manufacturing and labelling of egg products and egg protein-containing foods. In this case the labelling of foods containing additives known to cause allergenic reactions is an important point to consider. Therefore a considerable number of countries have introduced labelling directions for processed food products. However, these standards are not consistent and depend on the different regulatory status of the country of origin. Within the European Union egg proteins have been identified as food allergens and are included in the list of main allergenic substances in processed foods, Commission Directive 2003/89/EG and 2006/142/EG. A

detailed overview of the regulatory status for the European Union and the United States of America is given by the following references:

EFSA (2004): Opinion of the Scientific Panel on Dietetic Products, Nutrition and allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. Request No EFSA-Q-2003-016, adopted on 19 February 2004. *The EFSA Journal* 2004, 32: 1–197.

FDA (2006): The Center for Food Safety and Applied Nutrition, US Food and Drug Administration, US Department of Health and Human Services: Approaches to Establish Thresholds for Major Food Allergens and for Gluten in Food. Prepared by The Threshold Working Group, Revised March 2006.

Egg allergy is one of the most common food allergies in infants and young children. The vast majority is not life-threatening and management involves exclusion of egg from the diet and regular review with the expectation that the majority of children will outgrow the allergy by school age (Kemp, 2007). Most allergic reactions associated with egg involve the skin, but anaphylaxis also can occur. Elucidation of allergic reactions has shown that they are more frequently caused by egg white proteins than egg yolk. Approximately two-thirds of children diagnosed with food allergies are reactive to egg white (Mine, 2002). These findings are in agreement with the results of Anet *et al.* (1985). The authors showed that the main allergens could be found in egg white, but for a large proportion of the egg-sensitive patients, yolk contained specific IgE-binding constituents. Mine (2002) tested the binding activities of IgG and IgE antibodies from egg-allergic patients to physically or chemically treated egg white proteins. It was shown that the binding activities of purified egg white proteins against human IgE antibodies derived from egg-allergic patients with atopy followed the order ovomucoid > ovalbumin > lysozyme > ovotransferrin > ovomucin. To control compliance with the regulation directives and labelling requirements, analytical assays for the detection of egg in manufactured foods have been developed. Details for the performance of three commercially available kits for quantitative egg analysis can be found in Faeste *et al.* (2007).

7.11 References

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8

Soy proteins

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Abstract: Consumed for centuries in East Asia, soybeans have recently become popular in Western markets for the health benefits of their storage proteins, particularly due to the claim that they help reduce the risk of heart disease. This chapter investigates these storage proteins, which mainly consist of β-conglycinin and glycinin, and discusses their molecular structures, functions, relationships and subunits. The physicochemical properties and physiological functions of soy proteins as a food ingredient are examined and their nutritive value are investigated. Methods of improving soybean functionality and flavour through conventional breeding and genetic engineering are also considered.

Key words: soy proteins, soybeans, chronic disease, storage proteins, β-conglycinin and glycinin.

Note: This chapter was originally published as Chapter 6 'Soy proteins' by D. Fukushima in *Proteins in food processing*, ed. R. Y. Yada, Woodhead Publishing Limited, 2004, ISBN: 978-1-85573-723-5.

8.1 Introduction

For more than 2,000 years people throughout East Asia have consumed soybeans in the form of traditional soy foods, such as nimame (cooked whole soy), edamame (green fresh soy) (Fukushima, 2000a), soy milk (Fukushima, 1994), tofu (Fukushima, 1981), kori-tofu (freeze-denatured and dry tofu) (Fukushima, 1980 and 1994), abura-age (deep-fat-fried tofu) (Fukushima, 1981), sufu or tofu-yo (fermented tofu) (Fukushima, 1981 and 1985), soy sauce (Fukushima, 1985 and 1989), miso (Fukushima, 1985), natto (Fukushima, 1985), tempeh (Fukushima and Hashimoto, 1980), etc. In Western countries, soybeans had attracted people's attention in the 1960s as an economical and high-quality vegetable protein source for humans. In the United States, new soy protein products were developed, such as soy flour, soy protein concentrates, soy protein isolates, and their texturized products. These soy products were introduced into Japan at the end of the

Table 8.1 Consumption of traditional soy food products in Japan

	Soybeans[1]	Soybean meal[2]	Total
Tofu and its derivatives	496,000	0	496,000
Kori-tofu	28,000	0	28,000
Natto	128,000	0	128,000
Miso	162,000	0	162,000
Soy sauce	26,300	157,600	183,900
Soy milk	4,200	0	4,200
Major traditional products (Total above)	844,500	157,600	1,002,100
Non-traditional products (Soy proteins)		4,000 (as product)	4,000 (as product)
Food use total	1,032,000	401,000[3]	1,433,000[3]

[1] Shokuhin Sangyou Shinbunsha. [2] Ministry of Agriculture, Forestry, and Fisheries. [3] Including non-food meal other than feeds.

1960s, but their consumption remains only 40,000 metric tons as products. The major methods of consumption of soybeans in Japan are traditional, for which about one million metric tons of soybeans and soybean meal are used, as shown in Table 8.1. The manufacturing techniques and equipment for these traditional soy foods had made great progress through the technical innovations following World War II and the modernization of the manufacturing process had almost been achieved, by the end of 1980.

In Western countries, the history of soybeans for human consumption covers only several decades, where the non-traditional protein products described above are mainly used as ingredients in formulated foods for their functional properties, such as water and fat absorption, emulsification, foaming, gelation, binding, etc. These soy foods have penetrated steadily into Western countries as healthy foods, but the growth is not as high as was expected, perhaps owing to the strong off-flavors associated with the products. However, the consumption of soy foods in the United States has begun to increase abruptly with 1997 as a turning point (Liu, 2000). It is clear that this increase is due to the realization of the physiological properties which soybeans possess. Numerous investigations during the 1990s put soybeans in the spotlight, where soybean storage proteins and soybean minor components traditionally considered to be antinutritional factors have been recognized to have exciting roles in the prevention of chronic disease. Furthermore, the FDA confirmed the 'Soy Protein Health Claim' on 26 October 1999, that 25 grams of soy protein a day may reduce the risk of heart disease. The market is very much responsive to this health claim. Therefore, taking this opportunity, soy foods will penetrate rapidly into Western cultures and diets.

In the processing and utilization of soybeans, the following four points are very important. First is the nutritional and physiological aspects, second

is the functional properties working physicochemically in food systems, third is the unfavorable substances such as off-flavors, allergens, etc., and fourth is the creation of the beneficial cultivars. This chapter deals with the molecular structures and physicochemical functions of soybean storage proteins, the reevaluation of the nutritive value of soy proteins, the physiological effects of soy proteins and their fragments, the allergenic proteins in soybeans and the genetic improvements of soybean storage proteins, etc.

8.2 Soybean storage proteins: structure-function relationship of β-conglycinin and glycinin

Approximately 90% of the proteins in soybeans exist as storage proteins, which mostly consist of β-conglycinin and glycinin. β-Conglycinin (Koshiyama, 1965; Catsimpoolas and Ekenstam, 1969; and Koshiyama and Fukushima, 1976a) has the sedimentation coefficients (SC) of 7S, whereas glycinin (Mitsuda *et al*., 1965) has 11S. There are two kinds of globulins having the SC of 7S other than β-conglycinin, namely γ-conglycinin (Catsimpoolas and Ekenstam, 1969; Koshiyama and Fukushima, 1976b) and basic 7S globulin (Yamauchi *et al*., 1984). However, these two 7S globulins are minor components which account for less than a few percent. The major storage proteins, namely, β-conglycinin and glycinin, possess a variety of functional properties physicochemically for food applications as shown in the introduction. These functional properties are ascribed to the intrinsic physicochemical characteristics which are based on the molecular structures. Therefore, this section focuses on recent developments in the structure-function relationship of β-conglycinin and glycinin.

8.2.1 Basic structures of β-conglycinin and glycinin

β-Conglycinin is a glycoprotein and a trimer which consists of three subunits with a molecular mass of 150–200 kDa. Major subunits are α′, α, and β and their molecular weights are 72, 68, and 52 kDa, respectively (Thanh and Shibasaki, 1977). Besides these, there is a minor subunit called γ in β-conglycinin (Thanh and Shibasaki, 1977). The amino acid sequences of these subunits are similar to each other (Hirano *et al*., 1987). Each of the α′ and α subunits possesses one cysteine residue (-SH) near the N-termini, whereas the β subunit does not possess any cysteine residue (Utsumi *et al*.,
1997). No cystine residues (-SS-) exist in these subunits. β-Conglycinin exhibits molecular heterogeneity, where six molecular species are identified as $\alpha'\beta_2$, $\alpha\beta_2$, $\alpha\alpha'\beta$, $\alpha_2\beta$, $\alpha_2\alpha'$, and α_3 (Thanh and Shibasaki, 1978; Yamauchi *et al*., 1981). In addition, Yamauchi *et al*. (1981) found another species of β_3. β-Conglycinin trimers cause association or dissociation depending upon the pH and ionic strength of the solution (Thanh and Shibasaki, 1979).

Glycinin is a hexamer with a molecular mass of 300–380 kDa. Each subunit is composed of acidic (~35 kDa) and basic (~20 kDa) polypeptides, which are linked together by a disulfide bond (Staswick *et al*., 1984). In glycinin, five subunits are identified as $A_{1a}B_{1b}$ (53.6 kDa), A_2B_{1a} (52.4 kDa), $A_{1b}B_2$ (52.2 kDa), $A_5A_4B_3$ (61.2 kDa), and A_3B_4 (55.4 kDa), which are classified into group I ($A_{1a}B_{1b}$, A_2B_{1a}, $A_{1b}B_2$) and group II ($A_5A_4B_3$, A_3B_4) by the extent of the homology (Nielsen, 1985; Nielsen *et al*., 1989). Each subunit in group I has two cysteine and three cystine residues, whereas each subunit in group II has two cysteine and two cystine residues (Utsumi *et al*., 1997). Glycinin subunits exhibit polymorphism, that is, there are some amino acid replacements in the same kind of subunit among soybean cultivars (Mori *et al*., 1981; Utsumi *et al*., 1987). Moreover, glycinin exhibits molecular heterogeneity, because the molecule is a hexamer with a different subunit composition (Utsumi *et al*., 1981). Glycinin hexamers dissociate to their constituent polypeptides, subunits, and half-molecules, depending upon pH, ionic strength, and heating temperature (Wolf and Briggs, 1958; Mori *et al*., 1982).

8.2.2 The physicochemical properties of β-conglycinin, glycinin, and their subunits

In Table 8.2 are shown the properties of β-conglycinin, glycinin, and their subunits on the gel formation, thermal stability, and emulsification (Utsumi *et al*., 1997). The mechanisms on the gel formation of β-conglycinin (Nakamura *et al*., 1986) and glycinin (Mori *et al*., 1982; Nakamura *et al*., 1984) are studied in detail. Glycinin forms a turbid, hard, and not inelastic gel, whereas β-conglycinin forms a transparent, soft, but rather elastic gel, in 100 °C heating (Utsumi *et al*., 1997). The A_2 polypeptide of glycinin A_2B_{1a} subunit

Table 8.2 Functional properties of soybean storage proteins and their subunits working physicochemically in food systems[(1)]

Functionality	Proteins or subunits	Property or its difference
Gel formation	β-conglycinin	Transparent, soft, but rather elastic gel
	Glycinin	Turbid, hard, and not so fragile gel
	A_2B_{1a} subunit	A_2 polypeptide relates to gel hardness
	A_3B_4 subunit	A_3 polypeptide relates to gel hardness
	$A_5A_4B_3$ subunit	$A_5A_4B_3$ subunit relates to the easiness of gel formation
Thermal stability	Soybean storage proteins	β-conglycinin < Glycinin
	β-conglycinin subunits	$\alpha < \alpha' < \beta$
Emulsification	Soybean storage proteins	β-conglycinin > Glycinin
	β-conglycinin subunits	$\alpha \geq \alpha' >> \beta$

(1) Utsumi *et al*., 1997.

closely relates to gel turbidity, whereas the A_3 polypeptide of the A_3B_4 relates to the gel hardness. The hardness of glycinin gel increases in proportion to the content of A_3 polypeptide. The $A_5A_4B_3$ subunit relates to the easiness of gel formation, because of the easy cleavage of the hydrophobic bonds between the A_5 and A_4 acidic chains during heating. Further, the existence of A_4 acidic polypeptide raises the elasticity of the gel, and at the same time it makes the gel softer and more fragile (Lee *et al.*, 2002). *β*-Conglycinin is more unstable thermally than glycinin, but the emulsifying and emulsion-stabilizing abilities of *β*-conglycinin are much stronger than those of glycinin.

The physicochemical functions of proteins depend upon their three-dimensional structures substantially. The polypeptide chains of the protein molecules are unfolded through the heat treatment of soybeans and as a result the amino acid side residues buried inside a molecule are exposed on the surface. The exposed -SH or hydrophobic residues combine the protein molecules through -SH, -SS- interchange reaction or hydrophobic bonding, respectively. In this case, it is very important that these active residues are present at an accessible location of the molecules. Table 8.3 shows the numbers of -SH and -SS- groups in each subunit. The larger numbers of SH groups and their topology in glycinin make glycinin gel much harder and more turbid in comparison with *β*-conglycinin gel, whereas the higher hydrophobicity and more easily unfolded structure in *β*-conglycinin make its emulsifying ability much stronger than that of glycinin (Utsumi *et al.*, 1997).

8.2.3 The three-dimensional structures of *β*-conglycinin and glycinin

In order to improve these functional properties, it is necessary to know the theoretical relations between the functional properties and the three-dimensional structures of the molecules. The research on the three-dimensional structures of soybean storage proteins started 35 years ago. We

Table 8.3 Number of cysteine and cystine in each subunit of *β*-conglycinin and glycinin[(1)]

	Subunit	Cysteine (-SH)	Cystine (-SS-)
β-conglycinin	*α′*	1	0
	α	1	0
	β	0	0
Glycinin	$A_{1a}B_{1b}$	2	3
	A_2B_{1a}	2	3
	$A_{1b}B_2$	2	3
	A_3B_4	2	2
	$A_5A_4B_3$	2	2

(1) Utsumi *et al*., 1997.

investigated the three-dimensional structures of *β*-conglycinin and glycinin molecules through optical rotatory dispersion (ORD), circular dichroism (CD), infra-red absorption spectra, ultraviolet difference spectra, deutration studies, and so on (Fukushima, 1965, 1967, and 1968). However, the results obtained by these methods are indirect ones. For a direct and complete analysis of three-dimensional structures, soybean proteins must be crystallized, followed by X-ray analysis.

The complete amino acid sequence of molecular subunits of soybean storage proteins was determined in the early 1980s through the sequence analysis of full-length cDNA and a genomic clone (see the reviews of Fukushima, 1988, 1991a, and 1991b). For a long time, however, X-ray analysis of soybean proteins has not been carried out, because the molecular heterogeneities in both *β*-conglycinin and glycinin obstructed their crystallization. Utsumi's group has overcome these difficulties by using a special soybean variety, in which *β*-conglycinin molecules or glycinin molecules are composed of the same kinds of subunits. In the crystallization of *β*-conglycinin they used the soybean variety, of which *β*-conglycinin is composed only of *β* homotrimer, that is, 3*β*, whereas in the crystallization of glycinin, they used the variety of which glycinin is composed only of A_3B_4 homohexamer, that is $6A_3B_4$. Thus, they have succeeded in the crystallization and subsequent complete analysis of the three-dimensional molecular structures of both *β*-conglycinin and glycinin, as shown in Figs 8.1, 8.2, and 8.3 (Maruyama *et al*., 1999; Adachi *et al*., 1999; Fukushima, 2000b and 2001; Adachi *et al*., 2003).

This success should be recognized as epoch-making in the basic research of soybean proteins, because most of the properties of proteins are ascribed to the conformation of the molecular surface in the three-dimensional structures of the molecules. Furthermore, the elucidation of the detailed three-dimensional structures enables us to understand the theoretical

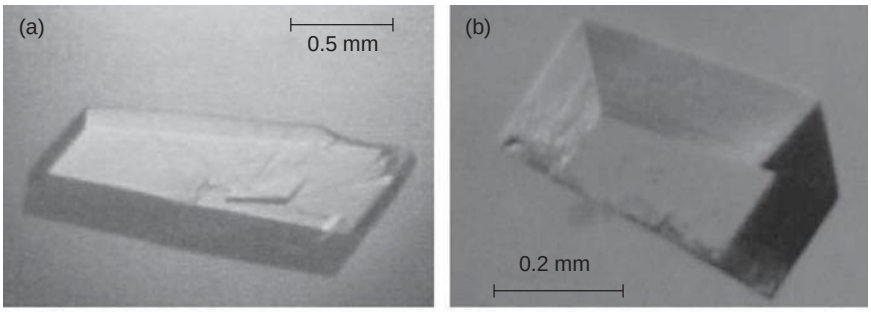


Fig. 8.1 The crystals of (a) *β*-conglycinin *β* homotrimer and (b) glycinin A_3B_4 homohexamer (Courtesy of Dr S. Utsumi).

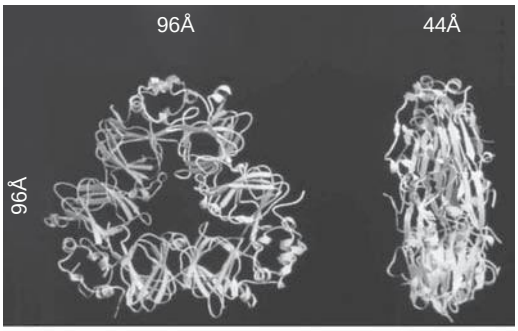


Fig. 8.2 Three-dimensional molecular structures of *β*-conglycinin *β* homotrimer (Courtesy of Dr S. Utsumi).

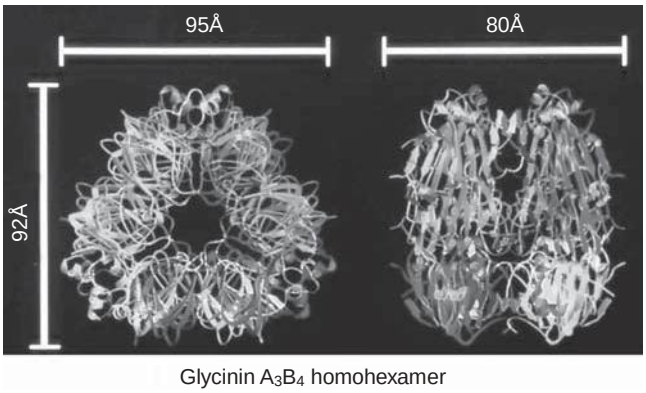


Fig. 8.3 Three-dimensional molecular structures of glycinin A_3B_4 homohexamer (Courtesy of Dr S. Utsumi).

modifications of the molecules, leading into the improvement of soybean protein properties at the genetic level. Table 8.4 shows the comparison between the X-ray data of Utsumi's group (Fukushima, 2000b and 2001) and our ORC and CD data (Fukushima, 1965, 1967, and 1968; Koshiyama and Fukushima, 1973) on the percentage of the secondary structures. It is very interesting that the results of X-ray analysis are in good accordance with the results of our indirect CD method around 30 years ago.

8.3 Soy protein as a food ingredient

8.3.1 Physicochemical properties of soy protein

It is generally known that soy protein ingredients have appropriate functional properties for food applications and consumer acceptability. These

Table 8.4 Contents of secondary structures contained in soybean storage protein molecules

	β-conglycinin		Glycinin	
	X-ray	CD[(1)]	X-ray	CD[(1)]
α-helix	10	5	8	5
β-structure	33	35	36	35
Disordered structure	57	60	56	60

[(1)] Circular dichroism.

functional properties are intrinsic physicochemical characteristics of soy protein, which affect the behavior of protein in food systems during preparation, processing, storage, and consumption. These properties are not only important in determining the quality of the final product, but also in facilitating processing, for instance improved machinability of cookie dough or slicing of processed meats. Physicochemical functions performed by soy protein preparations in actual food systems are solubility, water absorption and binding, viscosity, gelation, cohesion-adhesion, elasticity, emulsification, fat adsorption, flavor-binding, foaming, color control, etc.

As examples, gelation is important in comminuted meats, while emulsification and foaming are important in coffee creamers and dessert toppings, respectively. However, it should be noted that the physicochemical properties of the whole proteins contained in soybeans considerably differ depending upon soybean cultivars. As already described in Section 8.2.2, the physicochemical properties of each component of soybean proteins are quite different not only between β-conglycinin and glycinin, but also among the subunits of β-conglycinin or glycinin molecules. Further, both the ratio of β-conglycinin to glycinin and the subunit compositions of β-conglycinin or glycinin molecules are fairly different among soybean cultivars. This is the reason why the physicochemical properties differ among soybean cultivars.

Lee *et al*. (2002) compared the physicochemical properties of the heat-induced gels among the glycinin preparations produced from seven different soybean cultivars. Table 8.5 shows the relationship between the contents of A_1, A_2, A_3, A_4, and A_5, the acidic polypeptides of glycinin subunits, and the physicochemical properties of the gels. There were many differences in the gel textures among soybean cultivars. The glycinin gels were divided into two groups. One is the group of Shirotsurunoko, Hill, and York, which contains A_4 polypeptide and the other is Raiden, Suzuyutaka, Matsuura, and Yamabe, which lacks A_4 polypeptide. The former showed lower compressibility (CM), higher cohesive property (LC), and two to three times greater resiliency (RS) than the latter, indicating that A_4 polypeptide raises

Table 8.5 Relationships between the acidic polypeptide compositions and physicochemical properties of glycinin gels of seven soybean cultivars[1]

Cultivars	Acidic polypeptides				Mechanical parameters at rupture			
	A_1	A_2	A_3	A_4	F[2]	RS[3]	CM[4]	LC[5]
Shirotsurunoko	40.6	29.7	15.6	14.0	195.7	11.66	53.33	0.96
Hill	40.8	31.6	13.6	14.0	324.5	8.46	67.14	0.84
York	37.4	30.4	17.8	15.5	453.3	8.07	72.30	0.87
Raiden	42.7	32.0	25.3	Negl.[6]	410.3	3.74	83.33	0.67
Suzuyutaka	41.1	39.8	19.1	Negl.[6]	455.0	4.68	81.41	0.73
Matsuura	44.3	30.2	25.5	Negl.[6]	557.0	4.04	84.20	0.72
Yamabe-A3	40.0	34.4	25.7	Negl.[6]	685.3	3.85	88.10	0.63

[1] Lee *et al*., 2002. [2] Force. [3] Resiliency. [4] Compressibility. [5] Linearity of the compression process. [6] Negligible.

the elasticity of the gels and makes them softer and more fracturable. Among the gels of these seven cultivars, the most fracturable gel was of Shirotsurunoko and the most unfracturable gel was of Yamabe-A3. On the elasticity, the gel from Hill was the highest and that from Matsuura was the lowest. This indicates that the selection of the cultivars is important for the application of soybean proteins to food systems.

8.3.2 Reevaluation of nutritive value of soy protein

The quality of soybean proteins has actually been undervalued until recently, because the protein efficiency ratio was based upon the growth of laboratory rats. Growing rats not only possess a much higher requirement for proteins than infants, but also a much higher need for certain amino acids than humans (Steinke, 1979). Particularly, the rat requirement for methionine is about 50% higher (Sarwar *et al*., 1985). According to the Report of a Joint FAO/WHO/UNU Expert Consultation in 1985, the amino acid requirements are different depending upon human age, and methionine is not a limiting amino acid for soybean proteins, except in infants (see Table 8.6) (Fukushima, 1991a). Both the World Health Organization (WHO) and the United States Food and Drug Administration (FDA) adopted the protein digestibility corrected amino acid score (PDCAAS) method as the official assay for evaluating protein quality. Soybean proteins have a PDCAAS of 1.0, indicating that it is able to meet the protein needs of children and adults when consumed as the sole source of protein at the recommended level protein intake of 0.6 g/kg body wt. (Young, 1991). It is now concluded that the quality of soybean proteins is comparable to that of animal protein sources such as milk and beef.

Table 8.6 Patterns of amino acid requirements and soybean amino acid composition[1]

Amino acid (mg/g protein)	Pattern of requirement				Amino acid composition of soybeans
	3–4 Mo.	2–5 Yr.	10–12 Yr.	Adult	
His	26	19	19	16	27
Ile	46	28	28	13	48
Leu	93	66	44	19	78
Lys	66	58	44	16	61
Met + Cys	42	25	22	17	26
Phe + Tyr	72	63	22	19	90
Thr	43	34	28	9	35
Trp	17	11	9	5	13
Val	55	35	25	13	48
Total (including His)	460	339	241	127	426
Total (minus His)	434	320	222	111	399

[1] Joint FAO/WHO/UNU Expert Consultation (1985).

8.3.3 Physiological functions of soy protein

Formerly, soybean proteins had been considered to play a role only as traditional nutrients. In the latter half of the 1970s, however, it was found that soybean proteins have a hypocholesterolemic effect. As shown in Fig. 8.4 (Descovich *et al*., 1980), the serum cholesterol is lowered markedly when the animal proteins in the diet are exchanged with soybean proteins. Since then, numerous investigations on the hypocholesterolemic effect of soybean proteins have been carried out. According to a meta-analysis of 38 separate studies involving 743 subjects, the consumption of soy protein resulted in significant reduction in total cholesterol (9.3%), LDL cholesterol (12.9%), and triglycerides (10.5%), with a small but insignificant increase (2.4%) in HDL cholesterol (Anderson *et al*., 1995). In linear regression analysis, the threshold level of soy intake, at which the effects on blood lipids became significant, was 25 g. Thus, soy protein represents a safe, viable, and practical nonpharmacologic approach to lowering cholesterol.

It is clear that soybean storage proteins possess the hypocholesterolemic effect in themselves, because the plasma total cholesterol of the rats fed casein-cholesterol diets was reduced by 35 and 34% by the administration of purely isolated β-conglycinin and glycinin, respectively (Lovati *et al*., 1992). The exact mechanism of the cholesterol reduction has not been established fully. Some suggest that cholesterol absorption and/or bile acid reabsorption is impaired when soybean proteins are fed, while others propose that changes in endocrine status, such as alteration in insulin to glucagon ratio and thyroid hormone concentrations, are responsible (Potter, 1995).

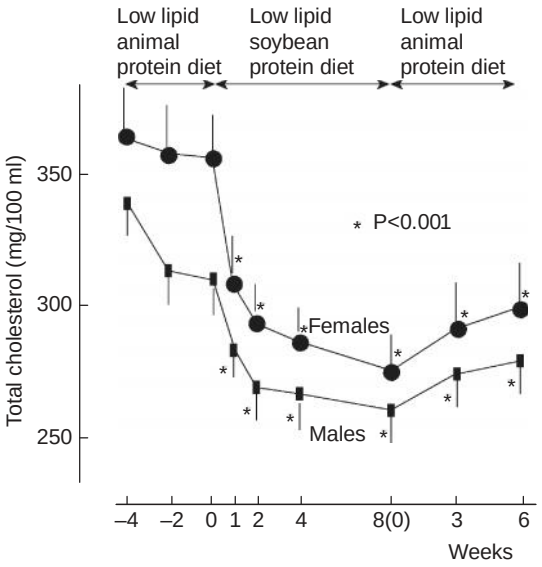


Fig. 8.4 Total cholesterol levels in type II patients treated with soy protein diets. * indicates highly significant difference from mean plasma lipid levels during the term before soy protein diets. Reprinted with permission from Elsevier Science (*The Lancet*, 1980, No. 8197, 709–712 by Descovich *et al.*).

In addition to the cholesterol-lowering effects described above, soybean proteins suppress the lipogenic enzyme gene expression in the livers of genetically fatty rats (Wistar fatty rats), indicating that dietary soybean proteins are useful for the reduction of body fats (Iritani *et al.*, 1996).

8.3.4 Physiologically active fragments derived from soybean storage protein molecules

Table 8.7 summarizes the physiological active peptide fragments derived from soybean proteins, which have hypocholesterolemic, anticarcinogenic, hypotensive, immunostimulating and/or antioxidant effects.

Iwami *et al.* (1986) found that the hydrophobic peptide fragments, which appeared through the proteinase digestion of soybean proteins, are responsible for their plasma cholesterol-lowering action. Since the hydrophobic peptides bound well to bile acids, the fecal excretion of bile acids is increased. Consequently, the bile acid synthesis in the liver is stimulated, resulting in the reduction of serum cholesterol. Soybean protein digests have the highest hydrophobicity among commonly used protein sources and give the lowest cholesterol level. Major hydrophobic peptides to bind to bile acids are A_{1a} and A_2, which are the acidic peptides of glycinin subunits, $A_{1a}B_{1b}$ and A_2B_{1a}, respectively (Minami *et al.*, 1990). The region comprising residues 114–161 (48 amino acid residues) represents the most

Table 8.7 Physiologically active peptide fragments from soybean proteins

Peptide fragments	Physiological activity	Protein source
High Molecular Weight Fraction[1]	Hypocholesterolemic and anticarcinogenic	Soybean proteins
LPYPR[2]	Hypocholesterolemic	Soybean glycinin
MLPSYSPY[3]	Anticarcinogenic	Soybean proteins
Peptide Fraction[4]	Hypotensive through ACE-inhibition	Soybean proteins
MITLAIPVNKPGR[2]	Phagocytosis-stimulating	β-conglycinin α'-subunit
MITLAIPVN[2]	Phagocytosis-stimulating	β-conglycinin α'-subunit
MITL[2]	Phagocytosis-stimulating and protection from hair loss	β-conglycinin α'-subunit
HCQRPR[5]	Phagocytosis-stimulating	Glycinin A_{1a}-subunit
QRPR[5]	Phagocytosis-stimulating	Glycinin A_{1a}-subunit
VNPHDHQN[6]	Antioxidant	β-conglycinin
LVNPHDHQN[6]	Antioxidant	β-conglycinin
LLPHH[6]	Antioxidant	β-conglycinin
LLPHHADADY[6]	Antioxidant	β-conglycinin
VIPAGYP[6]	Antioxidant	β-conglycinin
LQSGDALRVPSGTTYY[6]	Antioxidant	β-conglycinin

[1] Sugano *et al*., 1988. [2] Yoshikawa *et al*., 2000. [3] Kim *et al*., 2000. [4] Wu and Ding, 2001. [5] Yoshikawa *et al*., 1993. [6] Chen *et al*., 1995.

hydrophobic area of the A_{1a} subunit. This hydrophobic region is also highly conserved in the A_2 subunit.

Sugano *et al*. (1988) made the hydrophobic and high molecular weight fraction (HMF) resistant to microbial proteases, which exerts an extraordinarily strong hypocholesterolemic effect in rats through the increase in the fecal excretion of cholesterol and/or bile acids, compared to the parent soybean proteins. Most recently, however, Yoshikawa *et al*. (2000) found that Leu-Pro-Tyr-Pro-Arg, the low molecular weight peptide fragment derived from soybean glycinin, also reduced serum cholesterol in mice after oral administration. This may be a different mechanism from that of HMF, because there was no increase in the excretion of the fecal cholesterol and bile acids.

It is known that bile acid is an intrinsic promoter of colon cancer. Azuma *et al*. (1999, 2000a, and 2000b) and Kanamoto *et al*. (2001) found that HMF described above suppresses the tumorigenesis in the liver and colon in rats through the inhibitory effect on the reabsorption of bile acids in the intestine. Kim *et al*. (2000) discovered that Met-Leu-Pro-Ser-Tyr-Ser-Pro-Tyr, a low molecular weight peptide fragment derived from soybean proteins, has anticarcinogenic properties.

Using an *in vitro* assay exposes a variety of food-derived peptides which inhibit angiotensin-converting enzyme (ACE). *In vivo*, however, most of them did not show any antihypertensive effect. The peptides which showed

a real antihypertensive effect *in vivo* are only Ile-Lys-Pro from bonito muscle (Yokoyama *et al*., 1992), Ile-Pro-Pro and Val-Pro-Pro from fermented milk (Nakamura *et al*., 1995), and Ile-Lys-Trp from chicken muscle (Fujita *et al*., 2000). Many of the reported peptides were mere substrates of ACE and they showed apparent inhibitory activity by competing with a synthetic substrate which was used for the enzyme assay. Using a strong acid cationic exchange resin, Wu and Ding (2001) separated the peptide fraction from the enzyme hydrolysate of soybean proteins, which is composed of three peaks. The molecular weights and lengths of the peptides in this fraction were below 954 and between 2 and 8, respectively. This peptide fraction inhibited ACE and showed the hypotensive effect *in vivo*, when it was orally administrated on spontaneously hypertensive rats.

Immunostimulating peptides are expected to improve senile immunodeficiency. Yoshikawa *et al*. (2000) isolated a peptide stimulating phagocytosis by human polymorphonuclear leukocytes from soybean proteins. It is Met-Ile-Thr-Leu-Ala-Ile-Pro-Val-Asn-Lys-Pro-Gly-Arg which was derived from the α' subunit of β-conglycinin and named soymetide. Soymetide-4, the tetrapeptide at the amino terminus, that is, Met-Ile-Thr-Leu, is the shortest peptide stimulating phagocytosis. Soymetide-9 (Met-Ile-Thr-Leu-Ala-Ile-Pro-Val-Asn) is the most active in stimulating phagocytosis *in vitro*. Besides these, soymetide-4 prevents hair loss induced by a cancer chemotherapy agent. The peptides derived from soybean glycinin A_{1a} subunit, Gln-Arg-Pro-Arg and His-Cys-Gln-Arg-Pro-Arg, also stimulated phagocytotic activity of human polymorphonuclear leukocytes, but their activities are weaker than those of soymetide described above (Yoshikawa *et al*., 1993).

Chen *et al*. (1995) isolated six antioxidative peptides against peroxidation of linoleic acid from the protease hydrolysates of soybean β-conglycinin. They are

1. Val-Asn-Pro-His-Asp-His-Gln-Asn
2. Leu-Val-Asn-Pro-His-Asp-His-Gln-Asn
3. Leu-Leu-Pro-His-His
4. Leu-Leu-Pro-His-His-Ala-Asp-Ala-Asp-Tyr
5. Val-Ile-Pro-Ala-Gly-Tyr-Pro
6. Leu-Gln-Ser-Gly-Asp-Ala-Leu-Arg-Val-Pro-Ser-Gly-Thr-Thr-Tyr-Tyr.

These peptides are characterized by the hydrophobic amino acids such as valine or leucine at the N-terminal positions and proline, histidine, or tyrosine in the sequences. Yokomizo *et al*. (2002) also isolated four antioxidative peptides from the protease hydrolysates of the water-insoluble residues of soybeans. The amino acid sequences were

1. Ala-Tyr
2. Ser-Asp-Phe
3. Ala-Asp-Phe
4. Gly-Tyr-Tyr.

These peptides possess aromatic amino acid at the C-terminal end. Gly-Tyr-Tyr has the strongest antioxidative activity among these four peptides, which is nearly equal to that of carnosine. It should be noted that the molecular weights of these four peptides are much lower than those of the other antioxidative peptides previously isolated from soybean proteins (Chen *et al*., 1995), from Alaska pollack skin (Kim *et al*., 2001), and from fermented milk (Kudoh *et al*., 2001).

8.3.5 Physiological functions of soybean minor components

Another important factor in the physiological action of soybeans is that soybean minor components have exciting roles in the prevention of chronic disease (see Table 8.8). Although most of these minor components are not proteins, they coexist more or less in soy protein products as a food ingredient (Anderson and Wolf, 1995). Hitherto, these minor components, such as isoflavones, saponins, trypsin inhibitors, phytic acid, lectin, etc., were thought to be antinutritional factors, but now they are recognized to have preventative effects on cancer (Messina and Barnes, 1991). Among these, isoflavones (mainly genistein and daidzein) are particularly noteworthy, because soybeans are the only significant dietary source of these compounds. Isoflavones have not only anticarcinogenic activities, but also preventative effects on osteoporosis (Anderson and Garner, 1997) and the alleviation of menopausal symptoms (Albertazzi *et al*., 1998).

Table 8.8 Physiological functions of minor components contained in soybeans

Isoflavones	Anticarcinogenic activities[(1)], prevention of cardiovascular diseases[(2)], prevention of osteoporosis[(3)], antioxidant activities[(4)], and alleviation of menopausal symptoms.
Saponins	Anticarcinogenic activities[(1),(6),(7)], hypocholesterolemic effects[(6)], inhibition of platelet aggregation, HIV preventing effects (group B saponin)[(8)], and antioxidant activities (DDMP saponin)[(9)].
Phytosterol	Anticarcinogenic activities[(1)].
Phytic acid	Anticarcinogenic activities[(1),(6)].
Lectin (Hemagglutinin)	Activation of lymphocytes (T cell)[(8)] and aggregating action of tumor cells[(8)].
Nicotianamine	Inhibitor of angiotensin-converting enzymes[(10),(11)].
Protease inhibitors	Anticarcinogenic activities[(1),(6)].

[(1)] Hawrylewicz *et al*., 1995; [(2)] Setchell and Cassidy, 1999; [(3)] Anderson and Garner, 1997; [(4)] Yoshiki and Okubo, 1997; [(5)] Albertazzi *et al*., 1998; [(6)] Messina and Barnes, 1991; [(7)] Rao and Sung, 1995; [(8)] Harada, 1999; [(9)] Yoshiki and Okubo, 1995; [(10)] Kinoshita *et al*., 1993; and [(11)] Kinoshita *et al*., 1994.

Table 8.9 Major allergenic proteins in soybeans[1]

Protein assignment	Molecular wt. (kDa)	Frequency[2] (%)
Gly m Bd 30 k	30	65.2
Gly m Bd 28 k	28	23.2
α subunit of β-conglycinin	68	23.2
β subunit of β-conglycinin	45	10.1

[1] Ogawa *et al*., 1991. [2] Detection frequency among 69 soybean-sensitive patients with atopic dermatitis.

8.3.6 Off-flavors and allergenic proteins contained in soy protein

The most difficult problem limiting the expanded use of soy protein products in Western countries is the strong off-flavors associated with these products. There are two types of off-flavors. One is grassy and beany flavors and the other is bitter, astringent, and chalky flavors. The grassy-beany flavors are developed through the action of the three kinds of lipoxygenases 1, 2, and 3 present in soybeans. The bitter, astringent, and chalky flavors are caused by saponins and isoflavones (Okubo *et al*., 1992). The off-flavors of isoflavones are enhanced by the hydrolysis of their aglycones through the action of three kinds of β-glucosidases A, B, and C in soybeans (Matsuura and Obata, 1993). Thus, both lipoxygenases and β-glucosidases contained in soybeans play an important role in the production of off-flavors. Moreover, the lipid hydroperoxides produced by lipoxygenases oxidize the free -SH groups of soybean proteins, resulting in a decrease in their gel-forming ability (Fukushima, 1994). For a long time, a number of attempts have been made to remove or mask these off-flavors during processing. However, it has proved impossible to remove or mask the off-flavors to a satisfactory extent by these methods.

In addition to off-flavors, another unwanted characteristic in soybeans is allergenic proteins. The major allergenic proteins in soybeans are shown in Table 8.9 (Ogawa *et al*., 1991). It is noticeable that two of the three subunits of β-conglycinin have allergenic proteins. It is impossible to remove all of these major allergens through the usual treatments.

8.4 Improving soy protein functionality

8.4.1 Improvement through conventional breeding

In the last two decades, the various soybean mutant genes which control the production of enzymes, allergenic proteins, storage proteins, etc., have been identified in the world soybean germplasm. Using these mutants, commercially available soybean cultivars have been bred without having undesirable substances but with the beneficially modified composition of storage

proteins. For instance, the cultivar 'Kunitz' (Illinois Agricultural Experiment Station) lacking Kunitz's soybean trypsin inhibitor (Bernard *et al*., 1991) and the cultivar 'Ichihime' (Kyushu Agricultural Experiment Station) lacking all of the lipoxygenases 1, 2, and 3 (Nishiba *et al*., 1995) can be taken as examples. The development of a lipoxygenase-free cultivar will be beneficial for the production of non-traditional soy products, since Western people are very sensitive to beany flavors. However, the soybean cultivars lacking the β-glucosidases have not been developed yet, which are the enzymes enhancing the off-flavors by changing the isoflavones into their aglycones.

There was some progress recently on the removal of allergenic proteins. The cultivar with a high ratio of glycinin to β-conglycinin was developed by the group of Tohoku National Agricultural Experiment Station, named Tohoku 124. This cultivar lacks the two major allergenic proteins of 28 K and α subunit, while it still possesses allergenic proteins of 30 K and β subunit (Samoto *et al*., 1996). Fortunately, the 30 K protein can be removed easily by centrifugation, which is bound to IgE antibodies most strongly and frequently.

Another group of Kyushu National AES found the wild soybean line, named QT_2, which lacks all of β-conglycinin (Hajika *et al*., 1998). This line grows normally and produces successive generations, indicating the possibility of breeding the soybean varieties where storage proteins are mainly composed of glycinin without containing any β-conglycinin. Using this QT_2 line, they obtained the line lacking all the subunits of β-conglycinin by back-crossing with Fukuyutaka. This line contains only glycinin as storage proteins and it lacks the three major allergenic proteins of 28K, α, and β subunits (Takahashi *et al*., 2000). This performed as well in the field as Fukuyutaka in the on-campus experiment and was named Kyu-kei 305. Kyu-kei 305 should be mentioned as being the variety with the fewest allergens so far.

Besides these, the eight isogenic breeding lines with a different ratio of glycinin to β-conglycinin have been obtained by back-crossing, using Enrei as a recurrent parent in Nagano Chushin Experiment Station (Yagasaki *et al*., 1999). In each of these lines, not only the ratio of glycinin to β-conglycinin, but also the subunit composition of glycinin is varied systematically (see Table 8.10). The breaking stress of tofu gels made from the soybeans of these lines increases markedly with the increase of the contents of glycinin and its A_3B_4 subunit. The yields and protein contents of the soybean seeds in these lines are substantially the same as the parent Enrei, indicating the possibility of the breeding of practical cultivars, from which we will be able to produce a variety of soybean protein products with different physicochemical properties.

8.4.2 Improvement through modern genetic engineering

Now that the three-dimensional structures have been elucidated completely in the molecules of β-conglycinin and glycinin, it is possible to improve the

Table 8.10 Hardness of tofu gel made from soybeans with different ratio of 11S and 7S proteins and different composition of glycinin subunits[1]

Breeding line	Glycinin subunit			11S/7S in soy milk	Breaking stress of tofu gel
	Group I	$A_5A_4B_3$	A_3B_4		
Enrei (control)	+	−	+	58/42	9,891
EnB_2-111	+	+	+	66/34	9,989
EnB_2-110	+	+	−	62/38	8,955
EnB_2-101	+	−	+	57/43	10,171
EnB_2-100	+	−	−	45/55	7,162
EnB_2-011	−	+	−	52/48	6,791
EnB_2-010	−	+	−	33/67	4,835
EnB_2-001	−	−	+	25/75	5,381
EnB_2-000	−		−	12/88	3,002

[1] Yagasaki *et al*., 1999. Crops: Enrei (control), 380 kg; and others, 384–441 kg. Protein content of seeds: Enrei (control), 42%; and others, 39.3–40.7%.

qualities of soybean storage proteins both physiologically and physicochemically through genetic modification. For instance, Kim *et al*. (1990) made a modified $A_{1a}B_{1b}$ gene which has four additional methionyl residues near the C-termini. This modified $A_{1a}B_{1b}$ is excellent in both gelling and emulsifying properties. If this gene is introduced into the soybean lines lacking β-conglycinin such as Kyu-kei 305, the resultant transgenic soybeans are expected to have beneficial functional properties in food systems, together with a high content of methionine. We can also improve the properties of the storage proteins by introducing various kinds of physiologically active peptides into the molecules on the basis of the three-dimensional structures. In addition, transgenic rice (Momma *et al*., 1999) and potato (Utsumi *et al*., 1994) with glycinins have already been bred.

8.5 Conclusion

For a long time, the removal of off-flavors has been a primary concern in research and technology on the utilization of soy protein as a food ingredient. At present, however, the concern is changing to the physiologically active substances as well as the physicochemical functions of soy protein in food systems. For instance, isoflavones were considered simply as undesirable substances having strong off-flavors, but now they are recognized as being useful substances with an excellent preventative or alleviating effect on cancer, osteoporosis, and menopausal symptoms, etc. However, it should be remembered that the isoflavones are substances having both favorable and unfavorable properties for the development of new soy products.

The creation of new soybean cultivars is one of the most effective methods to obtain new soy protein products with beneficial characteristics, because the desirable or undesirable components of soybeans can be controlled essentially at a DNA level, through either conventional breeding or modern genetic engineering. The preventative effects of soy protein on chronic diseases have been proved scientifically and at the same time the relationship between the physicochemical properties and molecular conformations has been elucidated. Most recently, the three-dimensional molecular structures of both β-conglycinin and glycinin were cleared up completely, therefore the improvement of soybean cultivars through modern biotechnology will be continued extensively. The day is not far distant when ideal soybean cultivars for obtaining soy protein products as a food ingredient will appear.

8.6 References

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9

Peas and other legume proteins

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Abstract: This chapter discusses the potential and reality of using grain legumes, also known as pulses, as sources of proteins for human consumption. A number of methods exist for recovering proteins from ground seeds or flours and the method of isolation will affect the properties and potential use of these proteins. With a focus on pea proteins, processing of legumes to produce isolates, characteristics of the proteins in the isolates, as well as functionality and potential uses for these materials are included. The chapter will conclude with some of the challenges facing this industry.

Key words: legume protein, pulse protein, peas, beans, lentils.

9.1 Introduction

Dry field peas and other grain legumes, otherwise known as pulses, are the dry edible seeds of the pods of legume plants and are common throughout the world. In North America, some of the more commonly produced pulses include peas, beans, lentils and chickpeas. Some oilseeds such as lupines or soybeans are also considered to be closely linked to pulses. Although lupines will be discussed further in this chapter, information on soybean proteins can be found in Chapter 8.

Opportunity exists for the further development of pulses as ingredients as their use is currently limited in commercial food product applications. Commercially available ingredients include whole and dehulled flours, finely ground hull fibre from peas, air-classified protein and starch-rich flours, purified pea starch, pea protein concentrates and pea protein isolates. Peas are used most extensively as a source of commercial protein, fibre and starch over other pulses for several reasons. They are one of the more economically viable pulses to fractionate, they are grown extensively all over the world and the hull is easy to remove. It is for these reasons that

this chapter will focus mainly on pea protein; however, other pulse proteins which have typically been studied at an experimental level rather than a commercial scale will also be discussed. A summary of legumes that are potential sources of protein for human consumption is shown in Table 9.1.

Table 9.1 Summary of legumes commonly grown for food consumption

Latin name	Common names	Description
Pisum sativum	Split pea Field pea Dry peas	• Producers: Canada, France, China, Russia and the United States (Simsek *et al*., 2009) • Composition: 21–25% protein, 55–68% starch, 3.3–6.5% fibre, 1% fat, 2.4% ash (Aluko *et al*., 2009, Meiners *et al*., 1976) • Types include yellow peas, green peas, marrowfat peas (wrinkled pea)
Lens culinaris	Lentil Daal Dal	• Composition: 26.4% protein, 0.8% fat, 56.0% carbohydrate, 6.1% fibre, 2.6% ash (Meiners *et al*., 1976) • Common in Middle Eastern countries (Almeida Costa *et al*., 2006)
Cicer arietinum	Chickpea (Kabuli) Garbanzo bean Indian pea Chickpea (Desi) Konda	• Kabuli seeds are large (>0.3 g), have a smooth surface, and cream coloured (Saini and Knights, 1984, Wood *et al*., 2008) • Kabuli composition: 17.8–22% protein, 4.5–5.7% fat, 56.7–63% carbohydrate, 45% starch, 4.0–8.0% fibre, 1.9–3.2% ash (Meiners *et al*., 1976, Wang *et al*., 2010a) • Desi chickpeas small (0.1–0.3 g), more angular shaped, wrinkled surface and dark, thick seed coat (Saini and Knights, 1984, Wood *et al*., 2008) • Desi composition: 22.9% protein, 38.2% starch, 3.3% ash, 4.6% fat, 24.6% fibre (Wang *et al*., 2010a)
Phaseolus vulgaris	Navy bean White navy bean White pea bean Pea bean Haricot	• Popular in the United Kingdom and the United States (Lu *et al*., 1996) • Composition: 18.2% moisture, 21.1–24.5% protein, 1.5% fat, 39.0–56.3% carbohydrate, 6.6% crude fibre, 2.9–4.3% ash (Meiners *et al*., 1976, Wang *et al*., 2010a) • Dehulled flours fractionate into starch and protein-rich fractions (Aguilera *et al*., 1982)

Table 9.1 Continued

Latin name	Common names	Description
Phaseolus vulgaris	Pinto bean Mottled beans	• Common in the United States • Composition: 18.8–22.4% protein, 1.0–1.2% fat, 61.8% carbohydrates (42.5% starch), 6.3–18.9% fibre, 3.5–3.8% ash (Meiners *et al*., 1976, Wang *et al*., 2010a)
Phaseolus vulgaris	Small red bean Red Mexican bean	• Composition: 24.1% protein, 38.8% starch, 3.9% ash, 1.4% fat, 21.6% fibre (Wang *et al*., 2010a)
Phaseolus vulgaris	Kidney bean	• Consumed in Africa, India, Latin America and Mexico (Shimelis and Rakshit, 2007) • Composition: 21.5–27.1% protein, 1.1–1.2% fat, 61.7% carbohydrates (36.1% starch), 7.0%–20% fibre, 3.0–4.4% ash (Meiners *et al*., 1976, Wang *et al*., 2010a)
Phaseolus vulgaris	Black bean Black Turtle	• Common in cuisines of Latin America, southern United States and Spain • Composition: 32.9% protein, 38.8% starch, 4.2% ash, 1.7% fat, 21.2% fibre (Wang *et al.*, 2010a)
Phaseolus vulgaris	Cranberry Roman Speckled sugar	• Commonly used in Latin American, Italian, and Turkish cuisines • Composition: 24.0% protein, 39.5% starch, 4.1% ash, 1.3% fat, 14.6% fibre (Wang *et al*., 2010a)
Phaseolus limensis	Lima bean Haba bean	• Growing regions include Latin America, United States and Canada
Phaseolus lunatus	Madagascar bean	• Flours used as a food ingredient for functional and nutritional purposes • Composition: 20.4–22.3% protein, 0.8% fat, 62.1–63.8% carbohydrate (35–40% starch), 6.0–7.4% fibre, 3.4–4.2% ash (Meiners *et al*., 1976)
Vigna radiata *Phaseolus aureus*	Greengram Mung bean Green bean	• Common in Southeast Asia, Central Africa, China and United States (Walde *et al*., 2005) • Composition: 23.86–27% protein, 1.15% fat, 3.32% ash, 62.62% carbohydrates, 16.3% fibre, 6.60% total sugars, 9.05% water (El-Adawy, 2000)
Vigna mung *Phaseolus mungo*	Black gram Urad	• Common in Pakistan, Iran, India, Greece and East Africa (Shakoor-Chaudhray and Ledward, 1988) • Composition: 24% protein, 59.6% carbohydrate, 1.4% fat • Often dehulled and fermented (Senthil *et al*., 2006, Tiwari *et al*., 2007)

Table 9.1 Continued

Latin name	Common names	Description
Vigna unguiculata	Cowpea Catjang Yardlong bean Black-eyed pea	• Common in Sub Saharan Africa (Abu *et al*., 2005) • Good foaming ability; used in akara and moi-moi (Abu *et al*., 2005) • Composition: 9.7–11% moisture, 1.3% crude fat, 21.2–25% protein, 3.1–3.6% ash and 56.8–67% total carbohydrate (Abu *et al*., 2005, Giami, 1993, Kerr *et al*., 2000) and 3.9–6.0% fibre (Meiners *et al*., 1976)
Vicia faba	Fababean Broad bean Fava bean Field bean Horse bean	• Composition: 26.12% protein, 1.53% fat, 3.08% ash, 58.29% carbohydrates, 25% fibre, 5.70% sugar, 10.98% water (USDA, 2010) • Native to North Africa and southwest Asia, and extensively cultivated elsewhere • Used as a flour; eaten boiled or snacks
Cajanus cajan *Cajanus indicus* *Cytisus cajan*	Pigeonpea Red gram	• Cultivated legume in Nigeria and other tropical countries (Onimawo and Akpojovwo, 2006) • Composition: 5.14% ash, 21.32% protein, 7.29% fibre, 54.25% carbohydrates (Onimawo and Akpojovwo, 2006)
Lupinus augustifolius	Lupine Lupin	• Cultivated in Australia, Northern Africa and Western North America • Composition: 30% ash, 41.4% protein, 3.0% fibre, 7.6% fat, 3.5% starch (Sosulski and Youngs, 1979)

9.2 Processing and protein isolation

9.2.1 Dehulling

Pulses, the dry edible seeds of legume plants, are an important source of protein in many diets worldwide. Prior to consumption, pulse seeds may undergo several processing steps. Some of the more common processing methods include dehulling, soaking, boiling, pressure cooking as well as germination (Eyaru *et al*., 2009). Dehulling, considered to be one of the most important pulse post-harvest handling procedures, involves the removal of the high fibre, protective outer seed coat which surrounds the cotyledon (Wang, 2008; Sahay and Bisht, 1988; Wood *et al*., 2008; Tiwari *et al*., 2010). The hulls of pulses contain a significant amount of complex carbohydrate including 69% cellulose (Tosh and Yada, 2010) which contributes to total dietary fibre. When pulse hulls are removed the concentration of the protein present in the seed is increased since the hull component

contains little to no protein. The dehulling of pulse seeds also improves pulse seed appearance, texture, cooking quality, palatability and digestibility by removing the antinutritional components such as polyphenols and tannins (Egounlety and Aworh, 2003; Wang, 2005, 2008; Sreerama *et al*., 2009; Tiwari *et al*., 2007). Dehulling equipment can be subdivided into attrition style dehullers and abrasive dehullers, where the former can be used to remove loosely bound seed coats and the latter to remove tightly bound seed coats (Singh and Iyer, 1998). Some specific models of dehullers include stone chakki, under-runner disc sheller, cylinder and concave type dehullers as well as concentric cylinder type dehullers (Sahay and Bisht, 1988). Wood *et al*. (2008) recommended for chickpeas to be split in attrition style mills while Singh and Iyer (1998) recommended the abrasive style Satake mill as being useful for dehulling pigeon pea and field pea.

9.2.2 Air classification

Air classification is a physical technique that can be used to fractionate ground pulse crops into protein-rich and starch-rich fractions. Both whole and dehulled seed can be ground for use in this process where the lighter protein particles can be separated from the heavier starch particles in a stream of circulating air. The use of air classification in the processing of pulse crops received a lot of attention in the 1980s and much of the information gathered at that time is still in use today for the preparation of commercial air-classified protein fractions.

Many different legume crops have been subjected to air classification with varying degrees of success (Table 9.2). For most of the pulse flours evaluated, the percentage of the flour that ended up in the light protein fraction was between 20 and 40%. Dehulling prior to air classification does not affect the yield or protein content in the protein fraction. The high yield (82%) for lupine is a consequence of the high protein content (>40%) and low starch (~3.5%) in the flour (Sosulski and Youngs, 1979). By comparison, other pulses generally have less than 30% protein and more than 40% starch. Chickpea did not produce high yields, possibly due to the higher lipid content in the flour (~7%), most of which ends up in the protein fraction (Sosulski and Youngs, 1979).

The protein content of the resulting fraction is also an important consideration. Protein contents greater than 60% were achieved for a number of crops including fababean, horse bean, lentil, pea, navy bean and mung bean (Table 9.2). Despite the high yield for lupine, the protein in the resulting fraction was not much higher than was seen in the original flour. Chickpea also had low protein content in the protein fraction, possibly because of the influence of lipid on the separation process and the presence of lipid in the protein fraction. Oligosaccharides (Vose *et al*., 1976), phytic acid, hemagglutination activity and trypsin inhibitors (Elkowicz and Sosulski, 1982) all separate with the protein fraction and are present in much higher levels than in the original flour.

Table 9.2 Effectiveness of air classification of pulse crops

Crop	Yield of protein fraction	% protein in protein fraction	% starch in protein fraction	References
Chickpea	17–29	28.9–49.9	4.3–30.3	Sosulski and Youngs, 1979; Elkowicz and Sosulski, 1982; Sosulski *et al*., 1987[1]
Cowpea	25–29.2	44.9–51.6	3.2–11.2	Tyler *et al*., 1981[3]; Elkowicz and Sosulski, 1982; Sosulski *et al.*, 1987[1]
Faba bean	21.1–33.3	64.5–75.1	1.4–8.6	Sosulski and Youngs, 1979; Tyler *et al*., 1981[3]; Elkowicz and Sosulski, 1982; Tyler and Panchuk, 1982; Sosulski *et al.*, 1987[1]
Horsebean	28	66.1–69.0	4.2–7.3	Vose *et al*., 1976
Lentil	21.9–26	49.3–64.6	0.2–7.5	Sosulski and Youngs, 1979; Tyler *et al*., 1981[3]; Elkowicz and Sosulski, 1982; Sosulski *et al*., 1987[1]
Lima bean	23.8–27	40.4–49.6	0.0–8.2	Sosulski and Youngs, 1979; Tyler *et al*., 1981[3]; Elkowicz and Sosulski, 1982; Sosulski *et al*., 1987[1]
Lupine	82	43.3	–	Sosulski and Youngs, 1979
Mung bean	27.1–29	60.4	6.1	Sosulski and Youngs, 1979; Tyler *et al*., 1981[3]; Elkowicz and Sosulski, 1982
Navy bean	22.2–30.8	41.6–61.4	1.0–7.8	Tyler *et al*., 1981[3]; Silaula *et al*., 1989; Elkowicz and Sosulski, 1982; Zabik *et al*., 1983; Sosulski *et al*., 1987[1]
Northern bean	22.5–29.3	50.7–57.5	0.7–6.4	Sosulski and Youngs, 1979; Tyler *et al*., 1981[3]; Elkowicz and Sosulski, 1982
Pea	22.3–35.1	45.8–63.4	1.4–9.9	Vose *et al*., 1976; Sosulski and Youngs, 1979; Tyler *et al*., 1981[3]; Elkowicz and Sosulski, 1982; Tyler and Panchuk, 1982; Wright *et al*., 1984; Sosulski *et al*., 1987[1]; Wang *et al.*, 1999a
Pinto bean	Na	42.5	Na	Silaula *et al*., 1989
White bean[2]	32	53.8	Na	Sahasrabudhe *et al*., 1981

[1] Average from 3 different air classifiers.
[2] Average from 4 varieties.
[3] Data from first pass (series 1) only.

Particle size is critical to efficient separation in air classification as cell walls must be disrupted to a point where the protein and starch can be released (Tyler and Panchuk, 1982). A number of protocols have been developed to decrease particle size and improve yield, including multiple passes (Vose *et al*., 1976; Tyler *et al*., 1981; Wright *et al*., 1984), changing grinding speed (Wright *et al*., 1984), but the improved yield was generally accompanied by lower levels of protein. Different air classifiers produced fractions with different yields and protein contents (Sosulski *et al*., 1987) but again higher yields were accompanied by lower protein content. Yields were improved due to lower moisture during air classification but protein levels in the protein faction were decreased and starch levels increased (Tyler and Panchuk, 1982).

Overall, air classification can be used to produce protein-rich fractions from a range of legumes. However, the level of protein in this fraction is limited and the fraction will contain antinutritional factors such as oligosaccharides, phytic acid, hemagglutination activity and trypsin inhibitors. A protein concentrate produced through air classification is available from Parrheim Foods (Saskatoon, SK, Canada). This Prestige Protein which is derived from field pea, contains 50% protein ($N \times 6.25$, dry weight basis) and is reported to have excellent emulsification capacity, oil and water absorption and holding, and foaming capacity (Parrheim Foods, 2010).

9.2.3 Wet processing

While the level of proteins can be concentrated by air classification, the resulting products still contain an appreciable amount of starch (for most pulse crops) and some of the antinutritional compounds as noted above. As a result, alternate processing options, or further processing of the protein fraction from air classification have been examined.

Wet processing to purify proteins involves relatively few steps. The protein is extracted and separated from the residue and then the protein is recovered from the extract. There are, however, a range of solvents that have been used for extraction, and a number of different ways in which the extracted protein can be recovered. The extract can also be treated to reduce undesirable compounds and further increase protein content. While the value of 90% protein has sometimes been used to designate an isolate, some of the isolates prepared using this two-step approach with pulse proteins fall below this level. In this discussion, the term isolate will be used for all protein products recovered with this approach regardless of protein content.

Pulse protein extraction

The pulse proteins that are recovered for use as human food are storage proteins that are mostly globulins (Derbyshire *et al*., 1976), and it is these proteins that are extracted in the initial stage of protein isolation. One of

the most widely used approaches has been to use alkali in the extracting media and pH values ranging from 7.3 (Fredrikson *et al*., 2001) for peas to 12.0 (Sánchez-Vioque *et al*., 1999) for chickpeas. The choice of pH seems to vary with the crop and the researcher. It has been reported that use of alkali can result in reduced protein digestibility, an increase in the production of lysinoalanine, racemization of amino acids and a loss of quality in terms of functional properties (Swanson, 1990). As a result pH values are often selected to minimize these effects while achieving high yield.

For lentils, pH 8.5 to pH 9 have been used to recover the protein (Lee *et al*., 2007; Boye *et al*., 2010a) with yields ranging form 51 to 62% with protein levels at about 82% (Boye *et al*., 2010a). Dehulling prior to extraction did not improve yield. Beans are often extracted at a pH of 9.0 or higher (Sathe and Salunkhe, 1981b; McCurdy and Knipfel, 1990; Olivera-Castillo *et al*., 2007; Kaur and Singh, 2007a) with yields of 60–65% in isolates containing more than 90% protein (McCurdy and Knipfel, 1990). The high lipid content in chickpeas can be a problem in protein isolation and as a result, the starting material is usually defatted flour (Wang *et al*., 2010b; Kaur and Singh, 2007b; Singh *et al*., 2008; Sánchez-Vioque *et al*., 1999). By extracting at pH values between 8.5 and 12, yields of up to 82% with protein contents between 88 and 90% have been reported. By comparison, in the work of Boye *et al*. (2010a), where the flour was not defatted, extraction at pH 9.5 resulted in isolates with 64–77% protein.

Extraction of protein from peas generally starts with dehulled pea flour due to the ease or frequency in dehulling peas compared to some of the other pulse crops. The high protein fraction from air classification (Colonna *et al*., 1980; Sumner *et al*., 1981) or commercial protein concentrates (Shand *et al*., 2007) have also been used as the starting material. Extraction pH values of 9.5 (Boye *et al*., 2010a), 9 (Sumner *et al*., 1981), 8.5 (Vose, 1980; Shand *et al*., 2007), 8.0 (O'Kane *et al*., 2004a), 7.3 followed by adjustment to 8.5 (Fredrikson *et al*., 2001), and 7.0 (Colonna *et al*., 1980), have been used to produce pea protein isolates. A typical yield was ~83% protein with a protein content of 81–83% (Boye *et al*., 2010a; Shand *et al*., 2007), but protein contents of 92% protein were obtained with further processing to reduce oligosaccharides and phytate (Fredrikson *et al*., 2001).

Pea and other legume proteins can also be extracted using acid. For lentil, yields of 56–74% of the protein has been extracted at pH 2 (Fan and Sosulski, 1974), while Alli *et al*. (1993) used pH 4 to extract the protein from white kidney bean and Sathe and Salunkhe (1981a) were able to recover 81% of the protein in the acid extract with an additional 7% being recovered in a subsequent alkaline extract.

As the pulse storage proteins are primarily globulins, it is not surprising that salt has also been used to extract protein, although no improvement in the solubilization of fababean proteins was achieved by including 0.3 M NaCl during protein extraction at pH 7, 9 and 10 (McCurdy and Knipfel, 1990). A method where the initial extraction of protein uses NaCl without

pH adjustment was described by Murray *et al*. (1978; 1981) to minimize the changes in protein structure. When used in combination with a mild 'micellization' process to recover the protein, changes in the structure of fababean proteins (based on analysis by differential scanning calorimetry (DSC)) due to processing were minimal (Murray *et al*., 1985). This same approach has been used for extracting chickpea (Parades-López *et al*., 1991), lentil, pea (Bhatty and Christison, 1984), fababean (Bhatty and Christison, 1984; Murray *et al*., 1985) and pea protein (Sun and Arntfield, 2010).

Recovery of extracted protein

As the aim in preparing a protein isolate is to get as much protein in as pure a form as possible, the extracted protein can be treated to help in the purification through the removal of unwanted material. Fredrikson *et al*. (2001) compared two ultrafiltration membranes based on their abilities to reduce the oligosaccharide levels in the pea protein isolate and concluded the 100 kD membrane was more effective in reducing oligosaccharides (94%) than a 50 kD membrane (8%), though the protein content was higher in the isolate prepared with the 50 kD separation (92% compared to 89%). Paredes-López *et al*. (1991) increased the protein content in a chickpea protein isolate using a 10 kD membrane to concentrate salt extracted chickpea protein prior to precipitation. The addition of phytase effectively reduced the phytate level (Fredrikson *et al*., 2001). With these reductions in oligosaccharides and phytates, Fredrikson *et al*. (2001) simply adjusted the pH and dried the extract to get high protein isolates.

Ultrafiltration (50 kD membrane) as a method of protein recovery has also been used by Vose (1980) for alkaline pea and horse bean extracts and by Boye *et al*. (2010a) for alkaline extracts from peas, chickpeas and lentils. In both studies, the extracts were dried following the ultrafiltration step. When compared to proteins precipitated at the isolectric point from the same extracts, the isolates prepared with ultrafiltration had higher protein levels (Boye *et al*., 2010a) with the pea protein in the Vose (1980) study being an exception.

Another option for protein recovery is through protein crystallization. Using an acid extract from white kidney beans, Alli *et al*. (1993) left the extract at 4 °C for 18 h and recovered a crystalline precipitate. This approach has not been replicated with other pulse proteins.

For samples that have been extracted with salt, the 'micellization' process introduced by Murray *et al*. (1978) is an option. In this method the salt concentration is reduced by quickly diluting the extract in cold water or by dialysis. In response to this change in ionic strength, the proteins form micelles and precipitate. Bhatty and Christison (1984) used this process to prepare isolates containing 87, 91 and 95% protein for lentils, peas and fababean, respectively but found the nutritional quality of these isolates as the sole source of protein in a rat's diet was poor due to the low levels of sulphur amino acids and the presence of growth depressing factors (tannins,

trypsin inhibitors and hemagglutinins in the legume products). As 23% of the trypsin inhibitors remain in fababean protein isolate (Murray *et al*., 1985) this may have influenced rat growth in the Bhatty and Christison (1984) study. In a comparison of chickpea protein isolates prepared using isoelectric precipitation or by adding water at 4 °C (micelle formation promoted), a lower level of protein denaturation was seen with the micelle isolate (Parades-López *et al*., 1991). This process has also been applied to peas to produce an isolate containing 82% protein (Sun and Arntfield, 2010) and had a lower level of protein denaturation than a commercial protein isolate obtained from Nutri-Pea, Ltd (Portage la Prairie, MB, Canada). Enthalpy values of 16 J/g for the micelle isolate were obtained with DSC compared to 0.4 J/g for the commercial product.

While a variety of methods for recovering proteins from extracts have been investigated, the most widely used approach is to find the pH of minimum solubility and add acid (or alkali for an acid extract) to adjust the pH. For pulse proteins, precipitation pH values between 4 and 5 have been used (Vose, 1980; McCurdy and Knipfel, 1990; Sánchez-Vioque *et al*., 1999; O'Kane *et al*., 2004a; Kaur and Singh, 2007a; Lee *et al*., 2007; Olivera-Castillo *et al*., 2007; Singh *et al*., 2008; Boye *et al*., 2010a; Wang *et al*., 2010b), with the majority of them using pH 4.5. For alkaline extractions, yields are high with this approach, thus contributing to its popularity.

To increase the protein content in isolates, particularly when the protein has been recovered using isoelectric precipitation, washing steps can be included whereby the precipitate is rinsed (once or twice) with acidified water (McCurdy and Knipfel, 1990; Sánchez-Vioque *et al*., 1999; Kaur and Singh, 2007a; Sumner *et al*., 1981; Boye *et al*., 2010a; Wang *et al*., 2010b). Another way in which the protein resulting from isoelectric precipitation can be modified is by suspending the precipitate in water and adjusting the pH to 7, prior to drying. This will mean the product will have a neutral pH, which may be advantageous in some food applications.

For commercial isolates, information as to the isolation conditions used is not always available. The Propulse used by Nutri-Pea protein isolation is based on the acid extraction protocol originally developed by Nickel (1981). The Nutralys Pea protein is prepared by working with an aqueous dispersion from which the starch and fibre are removed (Fig. 9.1). Presumably this will help retain the native structure of the protein.

9.3 Characterization of pea and other legume proteins and isolates

Proteins are isolated to provide higher protein content and reduced levels of antinutritional factors. In addition, the amino acid composition is an important consideration when using pulse proteins to improve the nutritional value of a food product. Changes in protein structure can have an

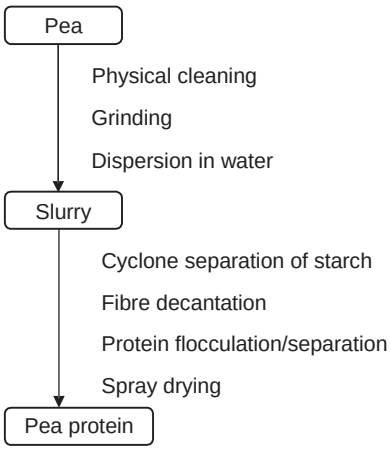


Fig. 9.1 Preparation of a pea protein isolation (commercial operation – adapted from data provided for Nutralys® pea protein by Roquette, 2010).

impact on protein functionality or the way in which they contribute to the properties of a food.

9.3.1 Isolate composition

As noted in the section on protein isolation, the protein level in the isolate can be highly variable. If the isolates from non-defatted chickpeas (64–77% protein, Boye *et al*., 2010a) are excluded, protein levels ranged from approximately 80 to 95% and the type of seed did not seem to be a factor in determining protein content. There were several treatments that tended to result in higher protein levels. The use of ultrafiltration, rather than isoelectric precipitation or precipitation by micelle formation, produced higher protein levels (Fredrikson *et al*., 2001; Boye *et al*., 2010a). This treatment should not only reduce some of the antinutritional factors, but also has the advantages of retaining both the albumins and the globulins, whereas the globulins are preferentially recovered when precipitating the protein. Sathe and Salunkhe (1981a) have reported that ~11% of the proteins in white beans are albumins. Washing the precipitated isolate also increased protein content, but yield was reduced (McCurdy and Knipfel, 1990). In a comparison of micelle formation and isoelectric precipitation, higher protein levels were obtained with the micelle isolate (Parades-López *et al*., 1991). The protein content in commercial pea protein isolates has been reported to be between 82 and 90%, reflecting the variations noted in experimental studies (Table 9.2).

Less emphasis has been placed on the antinutritional factors. As previously noted, Fredrikson *et al*. (2001) effectively reduced extracted oligosaccharides from 77 to 12 mg/g and phytates from 32 μ mole/g to less than

0.1 μ mole/g using ultrafiltration. The precipitation step was also effective in reducing antinutritional compounds. Working with an alkaline extract from cowpea, and precipitating at pH 4.33, Olivera-Castillo *et al*. (2007) demon- strated reductions of 60, 73, 79, 83 and 100% for phytates, trypsin inhibitors, tannins, α -amylase inhibitors and lectins, respectively. Using the micelliza- tion technique to precipitate the protein from a salt extract of fababean, the levels of trypsin inhibitors, phenols, hemagglutinin activity, phytic acid and vicine/convicine were reduced by 77, 88, 89, 91, and 95%, respectively, compared to the starting protein concentrate (Murray *et al*., 1985). It is clear that both precipitation methods significantly reduce the levels of antinutri- tional factors. While low levels of these factors will be present in most protein isolates, they are generally not of concern.

9.3.2 Protein structure and structural changes during isolation

As noted above, the proteins that are extracted are the albumins (water soluble) and globulins (salt soluble) and it is the globulins that tend to end up in the isolated protein. The albumins include the protease inhibitors, amylase inhibitors and lectins and their exclusion from the isolates is desirable (Boye *et al*., 2010b).

The globulins can be separated into two fractions, vicilin (7S) and legumin (11S). As similarities exist between the proteins in the various legumes, the vicilin and legumin from pea will be the focus of this discussion. Of the two proteins, there tends to be more variability in the vicilin fraction and, despite similar trimeric structures, vicilins can exhibit different surface, and therefore functional properties (Schwenke, 2001). Derbyshire *et al*. (1976) concluded there were two major proteins in the vicilin fraction with molecular weights of 150 000 and 190 000 and the former associated to an 11S form at low ionic strengths and pH values between 6.2 and 7. Croy *et al*. (1980) identified a third vicilin protein (convicilin) with a molecular weight of 290 000 (subunit molecular weight was 71 000). However O'Kane *et al*. (2004b) felt this was not a third vicilin protein, but represented the α -subunit of the second vicilin protein. Overall, it can be seen that the vicilin is a
heterogeneous fraction.

The pea legumin has a hexameric quaternary structure (Schwenke, 2001) with a molecular weight range of 300 000 to 400 000 (Derbyshire *et al*., 1976). It contains both acidic (high in glutamic acid) and basic (high in alanine, valine and leucine) subunits (Derbyshire *et al*., 1976). The subunits rely on areas of high hydrophobicity for interactions between subunits. Areas of high hydrophilicity on the surface of the molecule influence the solubility and interfacial properties of the protein (Schwenke, 2001). The 11S proteins are capable of dissociating to 7S and eventually 2S (acidic and basic) subunits, often in response to changes in pH (Derbyshire *et al*., 1976).

Protein isolates vary not just in terms of the amount of protein, but in the relative amounts of vicilin and legumin. This can be a result of the

genetics as well as the processing involved. This can result in differences in amino acid composition; an important consideration when legume proteins are promoted for their nutritional value. The amino acid contents (or partial contents) for three commercial protein isolates can be seen in Table 9.3. While there is some variability in the valine and glutamic acid content, the only essential amino acids that did not meet WHO standards (WHO/FAO/UNU, 2007) were the methionine + cystine combination and valine for one of the isolates. The low methionine + cystine level was not unexpected as legumes are known for having low levels of the sulphur amino acids. The variations in the biological value and protein digestibility corrected amino

Table 9.3 Characteristics and amino acid composition of commercial pea protein isolates

Property	FAO/WHO/UNU (2007) indispensable amino acid requirements	Propulse™ Nutri-Pea, Canada (Nutra-Pea, 2010)	Nutralys® Roquette Group, France (Roquette, 2010)	Pisane® Cosucra Groupe, Belgium (AB Ingredients, 2010)
% protein		82	80	88–90
Digestibility (%)		98	98	98
PDCAAS*		.98		.82
Biological value		78		53
Branched chain amino acids (%)		18	18	18
Histidine[1,2]	1.5	2.5	2.5	2.5
Isoleucine[1,2]	3.0	4.4	4.7	4.5
Leucine[1,2]	5.9	8.5	8.2	8.4
Lysine[1,2]	4.5	7.7	7.1	7.2
Methionine + Cystine[1,2]	2.2	1.5	2.1	2.1
Phenylalanine + Tyrosine[1,2]	2.5	9.7	9.3	9.3
Threonine[1,2]	2.3	3.8	3.8	3.9
Tryptophan[1,2]	0.6	0.8	1.0	1.0
Valine[1,2]	3.9	4.9	5.0	3.0
Alanine[2]		4.0	4.3	
Arginine[2]		8.2	8.7	
Aspartic acid[2]		12.0	11.5	
Glutamic acid[2]		18.5	16.7	
Glycine[2]		4.1	4.0	
Proline[2]		4.1	4.3	
Serine[2]		5.3	5.1	

* Protein digestibility corrected amino acid score = amino acid score × digestibility.
[1] Indispensable (essential) amino acid.
[2] All amino acids are reported as g/100 g protein.

acid score for these isolates may reflect differences in the availability of the amino acids for the isolates.

It has been noted that extraction and precipitation conditions can change the structure of proteins. For protein isolates, one of the more common techniques for looking at changes in protein structure is DSC, where the enthaphy of the transition (ΔH) is an indicator of the effects of prior processing.

A low ΔH value is an indicator of protein denaturation. Murray *et al.* (1985) followed structural changes in proteins isolated with a pH 8 extraction with isoelectric precipitation to those in proteins isolated with salt extraction and precipitated by micelle precipitation. In both cases the ΔH values were higher in the isolate than in the meal (~9 J/g) and the value for the isoelectric isolate (~14 J/g protein) was lower than for the micelle isolate (~24 J/g protein). The alkaline extraction was largely responsible for this difference. Lee *et al.* (2007) showed that as the pH of the extraction was increased from 8 to 9.5, the ΔH values for the protein in the isolate decreased from 11 to
7 J/g. The ΔH values for chickpea isolates (different chickpea varieties) extracted at pH 9 was only 4–6 J/g (Kaur and Singh, 2007a). With peas, Shand *et al.* (2007) report a ΔH value of 0.7 J/g in water for a pea protein extracted at pH 8, whereas Sun and Arntfield (2010) used a salt extraction, micelle precipitation technique to produce a pea protein isolate with a ΔH value of
15.8 J/g. The precipitation method can also make a difference. Using a salt extracted chickpea protein, Parades-López *et al.* (1991) reported a ΔH value of 3.9 J/g when isoelectric precipitation was used compared to 10.4 when the protein was precipitated through micelle formation. In studies using com- mercial pea protein isolates from Nutri-Pea, Canada, no ΔH values (Shand *et al.*, 2007) to a value of 0.03 J/g (Sun and Arntfield, 2010) have been reported. One can speculate that the conditions used for preparation of this commercial product were sufficient to denature the protein.

9.4 Functional properties in isolates and ways of improving them

Functional properties are determined to provide some indication of possible uses for a protein isolate. These properties relate to the way in which proteins interact with water, air, lipids and other proteins. If proteins do not function at the desirable level, attempts have been made to modify the protein or the chemistry of the test system to address these shortcomings.

9.4.1 Solubility

Protein solubility results from the hydrophilic groups on the surface of the protein interacting with water. The pH of the system is a major factor in

determining solubility, as solubility decreases around the isoelectric point of the protein. In general, most proteins have very low solubility between

pH 4 and 6, but outside this range, solubility increases dramatically reaching values up to 80–90% (Boye *et al*., 2010a; Sánchez-Vioque *et al*., 1999). It has been suggested that changes in the protein during precipitation decrease solubility and protein recovery using ultrafiltration should be more soluble (Schwenke, 2001). Higher solubility has been reported for ultrafiltered samples compared to those prepared by isoelectric precipitation, particularly in the pH range 4 to 6 (Vose, 1980; Boye *et al*., 2010a). The recovery of water soluble albumins with ultrafiltration contributes to this increase. The high solubility of these materials at pH 4 led to the suggestion that this might be a good source of protein for use in an acid beverage (Boye *et al*., 2010a). The use of micelle formation to precipitate chickpea protein was not as detrimental to solubility as isoelectric precipitation; solubility at pH 7 was 10% higher for the micelle isolate (Paredes-López *et al*., 1991). The specific proteins recovered in the isolate may also influence solubility; Koyoro and Powers (1987) found the vicilin was more soluble than legumin at all pH values examined.

Attempts to alter functional properties of legume proteins have not always helped the solubility. Tang and Ma (2009) found that a 30 min heat treatment at 95 °C increased solubility, but this was reversed with longer heating times, and Alonso *et al*. (2000) found the high temperatures used in extrusion cooking reduced the solubility of pea and kidney bean proteins. The replacement of the positive lysine with a neutral group through acetylation reduced the solubility of mung bean protein, whereas when succinlyation was used, and the lysine gained a negative charge, solubility was improved (El-Adawy, 2000). The complexing of pea protein and gum arabic resulted in a shift in minimum solubility to a lower pH resulting in a soluble complex at pH 4.2 (Liu *et al*., 2010).

Sosulski and McCurdy (1987) compared the solubilities of commercial pea and fababean isolates to soy protein isolates. The fababean and soybean proteins were produced by alkali extraction and the pea protein was extracted with acid. Both the fababean (40% solubility) and pea (38% solubility) were more soluble than soybean protein (31% solubility). While commercial pea protein isolate used in this study was not completely soluble (e.g. Propulse™ from Nutri-Pea Ltd has a protein solubility index of 15% at pH 7), it is reported to be easy to disperse (Nutri-Pea, 2010).

9.4.2 Emulsification

Emulsifying activity and stability are frequently measured when assessing protein functionality. The effectiveness of proteins as emulsifying agents is dependent on the surface properties and flexibility of the protein (Schwenke, 2001). As surface hydrophobicity has been correlated with emulsion activity (EA), emulsifying capacity (EC) and emulsion stability (ES), it is not surprising that protein isolation and modification techniques that impact surface properties will result in variations in emulsifying properties.

Many legume protein isolates, including those from pigeon peas, lima beans, yam bean (Akintayo *et al*., 1998), peas, fababeans (Sosulski and McCurdy, 1987) and chickpeas (Sánchez-Vioque *et al*., 1999) have been investigated as possible emulsifiers with varying results. With peas and fababeans, Sosulski and McCurdy (1987) found that the EC of a commercial pea isolate (acid extracted) and a fababean protein isolate prepared using an alkaline extraction and isoelectric precipitation were higher than the original flours, while chickpea isolates exhibited EC values lower than the original flour (Sánchez-Vioque *et al*., 1999). The pH used to make an emulsion has also been shown to influence the performance of these isolates. The stability of emulsions made with chickpea isolates was greater at pH 7 than at pH 4.5 (Singh *et al*., 2008). Increased exposure of hydrophobic and hydrophilic areas as the pH moves from the isoelectric point contributes to this observation. It is clear, therefore, that the ability of legume proteins to serve as emulsifiers depends on the legume used, as well as conditions used to form the emulsion.

The specific legume proteins (albumins, vicilin and legumin) differ in how they contribute to emulsion properties. The albumin fraction of Great Northern beans was more effective at incorporating oil into an emulsion than the globulin fraction (Sathe and Salunkhe, 1981b), yet the vicilin protein in the globulin fraction of peas had better EA and ES than the albumins due to the high surface hydrophobicity of the vicilin fraction (Cserhalmi *et al*., 1998). Regardless of the legume used, the legumin protein had inferior emulsion formation properties (Sathe and Salunkhe, 1981a; Dagorn-Scaviner *et al*., 1987; Koyoro and Powers, 1987; Cserhalmi *et al*., 1998), although differences in ES of vicilin and legumin were only seen at pH 3 (Koyoro and Powers, 1987). The poor performance of legumin was further reduced in the presence of small amounts of vicilin (Koyoro and Powers, 1987).

The method of protein isolation also affects emulsification properties. With alkaline extracted lentil proteins, both EA and ES decreased as the extraction pH increased from water to pH 9.5 (Lee *et al*., 2007). Isoelectric precipitation to recover pea proteins produced an isolate with higher EA (85%) than an isolate recovered by ultrafiltration (60%) (Vose, 1980) and both were lower than a soy isolate control (90%). However, when 4% NaCl was included in the emulsion, the EA for all three isolates was the same. Isoelectrically precipitated chickpea proteins also had higher EA than isolates prepared by salt extraction and precipitation by micelle formation (Paredes-López *et al*., 1991) although in this study both were higher than a soy protein control. It would appear that the mild extraction and precipitation techniques that attempt to retain protein conformation do not necessarily lead to good emulsion formation. Emulsion stability is a different story, as the ES ranking is often reversed in comparison to EC (Parades-López *et al*., 1991; Makri and Doxastakis, 2006). This inverse relationship may simply indicate that it is easier to maintain stability when the emulsion was not as well formed in the first place.

There have been several approaches to improve the emulsification properties of legume protein isolates and some are more effective than others. Limited exposure of hydrophilic groups using temperature (Tang and Ma, 2009) or pressure (Yin *et al*., 2008) improves both EA and ES, but this improvement was reversed with increased processing. Chemical modification of mung bean protein isolates resulted in an increase in EC, but only to limited levels of modification (El-Adawy, 2000); ES was also slightly improved. While conformational changes as a result of the modification of the lysine could expose hydrophobic groups, the balance between hydrophobic and hydrophilic groups may not have been ideal for emulsion formation. Complexing proteins with polysaccharides can also affect functional properties. When positively charged chitosan and negatively charged fababean legumin were combined, increased surface activity and greatly improved emulsion stability resulted, particularly at low protein concentrations (Braudo *et al*., 2001). Complexing the anionic arabinogalactan gum-arabic with pea protein resulted in a similar improvement in emulsion stability (Liu *et al*., 2010); this was even more effective if the protein gum arabic complex was well mixed prior to preparing the emulsion.

In general, legume proteins have great potential as emulsifying agents, and there are a number of techniques that can be used to further improve these properties.

9.4.3 Foaming

The ability of legume proteins to contribute to foam formation is also dependent on the surface properties. To create a foam, proteins must migrate and be absorbed at an air/water interface and then reorganize so that the surface tension of the air/water interface is reduced. To maintain a stable foam, the protein then needs to provide a viscoelastic film around the air bubble. The presence of hydrophobic and hydrophilic areas on the surface of the protein are again important for providing the optimal orientation of the protein at the interface, but unlike emulsification, the ability of proteins to produce stable foams is related to the 'exposable hydrophobicity' rather than the surface hydrophobicity (Schwenke, 2001). As a result, there needs to be more unfolding of a protein at an air/water interface than was needed for an oil and water system.

A number of different terms describe foaming properties but the volume of the foam created under standard conditions and how well that foam retains its structure over time are central to this property. As a result, in this chapter, foaming capacity (FC) will be used to refer to the increase in volume and foaming stability (FS) the change over time, regardless of the time frame used.

It is generally acknowledged that legume proteins can produce foams, and the FC can be increased by increasing protein concentration (Kaur and Singh, 2007a), but only to a certain level, after which the FC levels off (Sathe

and Salunkhe, 1981a). Isolation methods can influence foaming properties. Pea and horsebean proteins recovered from an alkaline extract using ultrafiltration had higher FC than commercial soy protein and skim milk powder (Vose, 1980), while proteins isolated through isoelectric precipitation had FC values below skim milk, but higher than soy protein. Foam stability was better than skim milk powder for both types of isolate. Pea and fababean isolates prepared by isoelectric precipitation had better foaming properties than soy protein, but the FC and FS values for the air-classified protein fractions, from which the isolates were prepared, were even better (Sosulski and McCurdy, 1987). The loss of low molecular proteins during protein isolation was thought to be responsible for the difference. The FC values were also significantly lower than that obtained for a commercial whipping protein, although the legume protein foams did retain more structure after sitting for two hours than the commercial protein.

The loss of albumins during most protein isolation procedures may be detrimental to foaming properties. Sathe and Salunkhe (1981a) reported that the albumins from beans had FC values that were twice those for the globulins, which had FC values similar to an egg white control. The values for protein isolates, however, were even lower. As a result it would be expected that the presence of albumins in an isolate that had been recovered using ultrafiltration rather than isoelectric precipitation would have better foaming properties. This proved to be the case in the work of Vose (1980) and Makri and Doxastakis (2006) where proteins were extracted at pH 8.5, but no differences were seen in the work of Boye *et al*. (2010a) where pH 9.5 extracts were used. Presumably the high pH used in the latter study had a greater impact on protein structure than the inclusion of albumins. Using lentil proteins, Lee *et al*. (2007) clearly demonstrated that conformational changes associated with increasing pH values used during protein extraction resulted in lower FC values, but higher FS values. Protein isolation using salt extraction and precipitation via micelle formation produced a chickpea isolate with lower FC and FS than an isolate prepared with alkaline extraction and isoelectric precipitation (Paredes-López *et al*., 1991). The specific legume globulins also differ in terms of foaming properties. The legumin protein from pea had a higher FC than vicilin, but the vicilin was found to produce a more stable foam (Koyoro and Powers, 1987). The lower content of disulfide bonds in vicilin provided the increased flexibility needed to maintain foam structure.

It is expected that modifications to the protein that enhance exposure of hydrophobic areas from the interior of globular proteins should improve foaming properties. The application of heat (95 °C) to kidney bean protein isolates, however, resulted in lower FC and slightly lower FS values, and longer heating time produced greater decreases (Tang and Ma, 2009). Aggregation of the protein, rather than exposure of hydrophobic areas resulted from this treatment. High levels of acetylation, where the positive charge on lysine is replaced by a neutral charge improved FC and FS by

unfolding the protein and increasing the overall hydrophobicity (Schwenke, 2001; El-Adawy, 2000). With succinylation, partial modification produced optimal results as the increase in the net negative charge at higher levels of modification affected the surface properties (Schwenke, 2001), despite causing changes in protein confirmation. The formation of a complex between pea protein and gum arabic had no effect on the FC (Liu *et al.*, 2010). However, the conformational changes in the protein were such that the stability of the foams formed between pH 3.1 and 4 were better than for the pea protein alone.

The potential for using pea and other legume proteins as foaming agents exists. Increased exposure of hydrophobic areas without promoting protein aggregation improves the performance of these proteins.

9.4.4 Gelation and film formation

Gelation of globular plant proteins is generally a heat-induced reaction in which protein aggregation follows heat denaturation. If protein-protein and protein-solvent interactions are balanced, a strong gel can form. However, if the protein-protein interactions predominate, a coagulum rather than a gel will result. Coagulums can result if the protein has a high percentage of hydrophobic residues (Schwenke, 2001) or if the time between denaturation and aggregation is shortened (Arntfield *et al.*, 1989). In a comparison of two vicilin components, O'Kane *et al.* (2004c) found one produced a much stronger turbid gel, while the other, which contained a highly charged N terminal region, formed a weak transparent gel. Electrostatic repulsion due to the charged area changed the characteristics of the gels.

Two approaches have been used to evaluate legume protein gels. Lowest gelation concentration (LGC) is based on the theory that if less protein is required to form a gel, it is a more effective gelling agent. The characteristic of the gels formed can be evaluated using techniques such as torsional rheometry (where stress and strain at failure reflect strength and elasticity) and oscillatory rheology (where the storage modulus, G', is an indicator of
gel strength and tan delta an indicator of elasticity).

For many protein isolates prepared using alkaline extraction and isoelectric precipitation (Kaur and Singh 2007a; 2007b; Sathe and Salunkhe, 1981a; O'Kane *et al.*, 2004a; Boye *et al.*, 2010a) or commercial isolates (Shand *et al.*, 2007; Sun and Arntfield, 2010), LGC ranged from 14 to 20%. Purified fractions or isolates prepared with alternative methods of protein precipitation generally had lower LGC values. For example, the LGC for an ultrafiltered chickpea isolate was 5.5% compared to 11% for the protein precipitated at the isoelectric point (Papalamprou *et al.*, 2009). This was attributed to differences in composition in that the isoelectric isolate was predominantly globulins, while the isolate from ultrafiltration contained both albumins and globulins. In the same study, an acid extracted ultrafiltered isolate, which was primarily albumins, had an LGC of 4.5%. Boye

et al. (2010a) also noted that LGC of isolates prepared by ultrafiltration had LGC values 2 to 4% lower than the corresponding isoelectric precipitates, and Sun and Arntfield (2010) obtained an LGC value for a micelle precipitate of a salt extract of pea protein of 5.5%, considerably lower than the 14.5% for a commercial pea isolate.

Gel properties of heat induced pea protein gels have been evaluated by Shand *et al*. (2007) and higher heating temperatures (92 versus 82 °C) were required to involve both legumin and vicilin in network formation. A commercial isolate (Propulse) produced stronger, less elastic gels than an isolate prepared in the lab using a pH 8.5 extract. Arntfield *et al*. (1991) showed that vicilin gels were weaker and less elastic than ovalbumin gels, presumably because of the increased involvement of disulfide bonds in ovalbumin gels. Sun and Arntfield (2010) also compared a laboratory micelle isolate to the Propulse commercial pea isolate and the micelle isolate was about ten times stronger and more elastic. Clearly the LGC and gel characteristics are very much dependent on the method of protein isolation.

Attempts have been made to improve legume gelation properties. Gel strength and elasticity were improved by the addition of microbial transglutaminase (MTG) (Shand *et al*., 2008). Using 19.6% pea protein and 0.7% MTG, a gel similar to one made from soy protein isolate was produced. In a gel structure containing pea protein, κ -carrageenan, and starch, the κ -carrageenan dominated the gelling behaviour and gels were not as strong as those from the commercial pea protein isolate (Nunes *et al*., 2006a).

Choi and Han (2001) demonstrated that a pea protein isolate/glycerol combination could be used to produce a film with properties equivalent to soy and whey proteins. Pea proteins also showed promise as microencapsulating agents. Using spray drying to encapsulate alpha-tocopherol, pea protein was able to retain 87% of the alpha-tocopherol. Although this was lower than the 97% obtained for a carboxymethyl cellulose/maltodextrin mixture, it was considered to be a successful application for pea protein (Pierucci *et al*., 2007).

While legume proteins can contribute to a number of properties, not all isolates perform in the same way. Commercially, there are several pea protein isolates available and a summary of three of them and their suggested functional properties and applications is shown in Table 9.4.

9.5 Utilization of pea and other legume proteins in foods

Isolation of pulse protein (most often peas and lupines) enables the protein to be used in a variety of applications. Apart from their versatility as ingredients, pea proteins provide a positive perception in terms of health benefits, such as high digestibility (protein digestibility corrected amino acid score of 0.69 has been reported; Avila, 2008), and low allergenicity (Avila, 2008). As the functional properties varied with the method of isolation, so will the

Table 9.4 Functional properties and recommended uses of commercial pea protein isolates

Property	Propulse™ Nutri-Pea, Canada (Nutra-Pea, 2010)	Nutralys® Roquette Group, France (Roquette, 2010)	Pisane® Cosucra Groupe, Belgium (AB Ingredients, 2010)
Solubility	Low (5 to 20% depending on pH) Good dispersibility Use in beverages	Highly soluble Very dispersible	Use in vegetable based shakes
Emulsification	No information available	Good emulsion capacity and stability	No information available
Foaming	No information available	No information available	No information available
Gelation/film formation	Strong and stretchable films Microencapsulation	Firm gel Good for meat formulations	No information available
Others	No information available	Excellent for water and fat binding	No information available

applications. Applications can range from ingredients in traditional product to use in novel food product formulations as well as in films or microcapsules. The common food use applications include beverages, bars, processed meats, desserts, dairy style products, sauces and baked goods (Karleskind *et al.*, 2004). These applications are discussed in greater detail below.

9.5.1 Beverages

Innovation in the beverage market is extensive with nutritionally dense ingredients such as proteins replacing calorie dense substances such as sugar. Novel beverages include smoothies, infant formulas, fruit juices, yogurt drinks, sports endurance drinks, carbonated beverages, slushes and frozen beverage mixes in which protein acts as a nutritional or functional ingredient. However, it may be necessary to add other components such as starch, cellulose, carrageenan, pectin, and gelatin to achieve the desired texture and stability (Karleskind *et al*., 2004).

Karleskind *et al*. (2004), while working primarily with soybean, cited lupine as an appropriate protein source for use in beverages and this protein source was used by Snowden *et al*. (2007) to create a dairy protein substitute, milk-style beverage. Using proteins precipitated by micelle formation, Murray *et al*. (1983) indicated field pea, chickpea, fababean, navy bean and pinto bean isolate could be used to prepare a neutral protein beverage resembling a dairy beverage similar to milk which could be served hot or

cold. The protein isolates used in these applications were added at levels of 0.1–10% (by weight) of the mix.

To form an acceptable beverage, ingredients used must be dispersible and capable of forming a homogeneous suspension; the settling of solid particles within the suspension is undesirable. Colour and brightness of ingredients are also important traits to consider as browned ingredients are less desirable than brighter white colours. In applications such as milkshakes the ability to foam is desirable, but this trait may be undesirable in other applications (Karleskind *et al*., 2004). The lupine protein described by Snowden *et al*. (2007), when correctly extracted and processed, embodies these positive characteristics. The proteins were isolated by alkaline extraction (pH 8 to 10) and precipitated in the pH range 3.0 to 5.5. The proteins were then resolubilized to make the milk-like product. Removal of low molecular weight proteins attributed to flavour issues such as bitterness and off flavours was achieved using ultrafiltration (Karleskind *et al*., 2004). Murray *et al*. (1983) indicated their product was stable, with a non-objectionable taste, low viscosity and having a high protein concentration.

A blended vegetarian protein product was invented by Avila (2008) which incorporated soy protein, rice protein and pea protein (in equal amounts) as a nutritional supplement with a protein digestibility similar to whey that was recommended for use in protein shakes with favourable flavour and texture. In a sensory evaluation, it was found that 7/10 consumers preferred the rice/soy/pea protein shake to an all soy protein shake. It was felt that this blended protein product could be used in other products including bars, drink mixes, tablets, wafers, liquids, spray nutritionals, soft gels and chewable tablets (Avila, 2008). The pea protein isolate Propulse, from Nutri-Pea Ltd has also been marketed based on its use in beverages. A recipe for using this material is shown in Table 9.5.

9.5.2 Gluten-free applications

Approximately 1% of North Americans express intolerance to gluten proteins and since 2001, the market for gluten-free products has grown by 27% (Han *et al*., 2010). Presently, many gluten-free foods contain high concentrations of starch, lack nutrients and have a high glycemic index (Fontanesi and Budelli, 2007). The addition of gluten-free proteins may help to achieve a higher nutritional profile for these products.

Gluten-free cracker formulations were developed by Han *et al*. (2010). A pea protein cracker demonstrated good crispiness and, of the crackers tested, was most frequently preferred and had the highest overall acceptability score in part due to the high scores for colour and flavour. Nutritionally balanced, low glycemic index gluten-free pasta was invented by Fontanesi and Budelli (2007) incorporating non-gluten starch, flour, protein and emulsifiers. Recommended starches were potato or maize, and flours

Table 9.5 Formulation for a beverage using pea protein isolate[1]

Product name: Equipped
Description: Low fat chocolate fortified beverage containing pea protein, vitamins and minerals

Ingredient	Percent of formulation
Water	82.12
Sugar, granular	7.00
Pea protein isolate – Propulse	4.20
Non-dairy creamer – 505 fat	3.60
Cocoa, 10/12 RDP	1.20
Vitamin/mineral blend	1.16
Soy Mask Flavor Nat # NV24 261	0.30
Disodium phosphate	0.20
Vanillin	0.10
Pectin, low methoxyl, amidated	0.06
Xanthan gum, Insta Thick	0.06

Procedure:

Prepare pectin/xantham gum solution using water at 82–93 °C (180–200 °F). Mix 3 min to hydrate gums. Add remaining ingredients and mix for an additional 5 min.

Heat mixture to 65–71 °C (150–160 °F), pass through a vacuum decanter. Homogenize at 95 KPa (2000 psi) [1st stage @ 72 KPa (1500 psi), 2nd stage @ 24 KPa (500 psi)].

After homogenization, pass through a plate heat exchanger to achieve 75 °C (167 °F) with a hold time of 15 sec. Then cool to 4.5 °C (40 °F) before filling under sanitary conditions.

Keep product refrigerated.

[1] Information provided by Nutri-Pea Limited, Portage la Prairie, MB, Canada (Nutri-Pea, 2010).

and protein could be from pea, bean, broad bean, soy, carob, lentil, peanut, lupine or mixtures of these materials. The concentration of protein in the formulation was recommended at 2–8%. With the proper combination of ingredients, Fontanesi and Budelli (2007) claimed that the glycemic index was 27, half of that for regular pasta and a third of the glycemic index of rice pasta. The gluten-free pasta was also described as having excellent organoleptic properties, and excellent cooked texture.

Gluten-free flours can also be used for baking, though often the pea proteins are used in combination with other protein sources. Marco and Rosell (2008) combined a commercially available pea protein isolate with rice flour and soybean protein isolates and incorporated the enzyme trans-glutaminase to aid in the development of a cohesive protein network. When formed into dough, all three proteins worked together to form a compact structure. Using a combination of corn starch, amaranth flour (0–40%), pea protein isolate (1–6%) and psyllium fibre (0–2%), Mariotti *et al*. (2009) created a gluten-free nutritional flour. When worked into a dough, the

elastic and loss moduli indicated that the gluten-free dough demonstrated solid elastic-like behaviour.

9.5.3 Meat product applications

Vegetable proteins are often used in meat product applications as extenders of comminuted meat products like sausages and patties. They can also be used to mimic the texture of meat as vegetable protein meat analogs.

The addition of a lupine protein extract to a meat batter formulation at a level of 2% improved the processing and cooking behaviour of the product and also enhanced the final product quality (Drakos *et al*., 2007) of comminuted meats and emulsion gels. Overall, the lupine proteins contributed by strengthening the structure in the meat gel system. A formulation for chicken sausages prepared using the Nutralys® pea protein isolate from Roquette is provide in Table 9.6.

Meat analogs resembling the texture of meat protein such as chicken, beef, pork or seafood were created from vegetable proteins, including pea protein, by processing extracted protein through a cooking extruder to create long fibrous strands of protein (Morimoto *et al*., 1982). Further rehydration, shredding and shaping of the fibres produced a protein analog which contained 30–100% heat coaguable protein, 0–17% non-heat coaguable protein, 0–30% starch and 0–2.5% sulphite. Extrusion texturization of an air-classified pea protein fraction (55% protein) at 170 °C was used by Wang *et al*. (1999a) to give a texturized pea protein whose brightness was similar to a texturized soy flour but less than a texturized soy protein.

9.5.4 Desserts and dairy

Vegetable proteins that exhibit good gelling, emulsifying, fat absorbing and water binding properties can be effective in the creation of gelled desserts. Nunes *et al*. (2006b) used pea protein (0–4%), κ -carrageenan (0–0.30%) and starch (0–5%) to formulate a gelled vegetable dessert suitable for those who wish to avoid the consumption of animal-based dessert products for health or ethical reasons. The excellent gelling characteristics of the pea protein isolate make this ingredient a suitable replacement for egg and milk proteins (Nunes *et al*., 2006a). The firmness level of the pea protein isolate gel was similar to that of soy protein gels but much lower than that of dairy protein desserts; however, the texture with pea protein isolate was closer to dairy desserts than with soy protein isolate. Gelled desserts were also prepared using pea or lupine protein isolates (2%) with κ -carrageenan or gellan (0.15%), xanthan gum (0.2%), starch (2.5%) and sucrose (15%) (Nunes *et al*., 2003).

Frozen desserts can also be formulated with vegetable protein ingredients. Using lupine as an emulsifier and a protein source, Eisner *et al*. (2008;

Table 9.6 Formulation for a meat product that includes pea protein isolate[1]

Product name: Chicken sausages
Product description: Chicken sausage containing pea protein (Nutralys® F85M)

Ingredient	Percent of formulation
Breast and leg of chicken	32.84
Vegetable oil	19.31
Water/ice	38.15
Nitrite salt	1.64
Phostabyl	0.39
Pea Protein – NUTRALYS® F85M	1.45
Modified starch CLEARAM® PGHV	4.85
Delispice	0.97
HVP	0.19
Erythorbate	0.04
Hemoglobin	0.19

Procedure:

Prepare Mix 1 by combining the nitrite salt, the phostabyl and the pea protein.
Prepare Mix 2 by combining the modified starch, delispice, HVP, erythorbate and hemoglobin.
Prepare a blend with cold water (4 °C) and crushed ice.
In a Stephan cutter, under vacuum, add ingredients using the following times and cutting speeds.

	Time	Speed	Vacuum
Mix 1 + chicken	0	0	No
½ water/ice	2 min	1500 rpm	No
Vegetable oil	3 min 20 sec	3000 rpm	No
Mix 2	4 min 10 sec	3000 rpm	Yes
½ water/ice	5 min	3000 rpm	Yes ($T < 14\,^{\circ}C$)
End	7 min	3000 rpm	Yes

Form the sausages.
Cook 20 min at 55 °C and 30% RH.
Smoke 20 min at 55 °C and 30% RH.
Cook with a stove at 75 °C and 100% RH to an internal temperature of 72 °C.

[1] Information provided as a guideline by Roquette Freres, Lestrem cedex, France (Roquette, 2010).

2009) formulated ice cream style products that could be marketed as a non-genetically modified frozen dessert. Lupine protein incorporated at 3–15% of the product formulation was paired with flavours such as strawberry, raspberry, cherry, chocolate and vanilla. Snowden *et al.* (2007) has indicated that the white opaque colour, smooth texture, neutral aroma and emulsifying and binding properties of this protein make it an ideal ingredient in food product formulations requiring the replacement of dairy proteins such

as for yoghurts, ice-cream and cheese style products where the lupine protein is incorporated at levels ranging from 1 to 10%.

9.5.5 Cereal-based products

The addition of pulse protein to cereal food products is generally done to improve the protein content and nutritional quality of the products due to the relatively high concentration of lysine present in pulse protein compared to cereal proteins (Lorimer *et al*., 1991; Zabik *et al*., 1983; Fleming and Sosulski, 1978; Spink *et al*., 1984; Hsu *et al*., 1982). By using these complementary proteins, worldwide protein insufficiencies in diets due to limited availability or ethical constraints associated with the consumption of animal proteins can be addressed (McWatters, 1980; Silaula *et al*., 1989; Boye *et al*., 2010b). The high protein fractions from air classification of peas, lentil, fababean, pinto bean, navy bean, cowpea and black bean have been used in these applications. These fractions contain protein levels ranging from 39.3 to 61.1% (Fleming and Sosulski, 1977; Aguilera *et al*., 1982; Zabik *et al*., 1983; Spink *et al*., 1984; Silaula *et al*., 1989). Ideally, protein-rich legume flours are combined with wheat flours in amounts sufficient to make protein nutrient content claims, but with increased substitution of the pulse flour, product quality in terms of texture, colour and flavour is typically compromised. Concentrations of 5–20% pea protein (Fleming and Sosulski, 1977; Zabik *et al*., 1983; Lorimer *et al*., 1991; Silaula *et al*., 1989) have been added to the formulations of wheat breads; however, the reduction in the gluten protein of the bread causes a reduction in the quality of dough and bread by disrupting the cohesive protein/starch network (Fleming and Sosulski, 1978). Other detrimental effects include delayed dough development times, decreased dough stability (Silaula *et al*., 1989; Lorimer *et al*., 1991), reduction of loaf volume, lower specific volume, and changes in crumb grain, crumb compressibility, and loaf shape (Fleming and Sosulski, 1977). Vital gluten, dough stabilizers, conditioners and surfactants can be added to improve the texture of breads fortified with protein-rich legume flours (Fleming and Sosulski, 1977; Silaula *et al*., 1989). Biscuits, cookies and doughnuts have also been used as a medium for the delivery of high protein pulse flours as 10–30% of the formulation (McWatters, 1980; Zabik *et al*., 1983; Spink *et al*., 1984). As these products rely less on the ability to retain gas in the crumb texture than breads, a higher concentration of pulse protein flours can be included in the formulation of the product.

9.5.6 Commercial pulse protein sources

In addition to the inventions and research on utilization of legume proteins described above, a number of products have been made with commercially available protein material, the results of which are summarized in Table 9.7.

Table 9.7 Summary of products made using commercial protein products

Product	Commercial protein source	Reference
Gluten-free cracker applications	Nutri-pea pea protein	Han *et al*., 2010
Gluten-free pasta and dough	Lupin protein concentrate Lupidor P052-H500 Naprofood GMBH & Co	Fontanesi and Budelli, 2007
Protein enriched gluten-free composite flours	Pea protein isolate Trades SA (Barcelona)	Marco and Rosell, 2008
Gluten-free doughs	Pea protein isolate Pisane® F9 Prodohi Gianni SpA (Milan)	Mariotti *et al*., 2009
Gelled vegetable desserts	Pea protein isolate Pisane® Cosucra Belgium	Nunes *et al*., 2006b
Vegetable proteins and milk puddings	Pea protein isolate Pisane® Cosucra Belgium	Nunes *et al*., 2003
Comminuted meat and emulsion gels	Lupin seed protein isolate LSPI type E Fraunhofer Institute	Drakos *et al*., 2007
Vegetable proteins and milk puddings	Commercial lupin isolate LupiE Fraunhofer Inst	Nunes *et al*., 2003
Extrusion texturization of air-classified pea protein	Air-classified pea protein Parrheim foods	Wang *et al*., 1999a,b
Extrusion of granulated pea proteins	Pea protein isolate Pisane® HD Cosucra and Propulse™ Parrheim foods	Boursier *et al*., 2008

9.6 Future challenges and trends in using peas and other legume proteins

Although there are nutritional advantages to using legume proteins in food product formulations, the use of these ingredients remains limited in commercial applications. Considering the novel nature of these proteins as ingredients, consumers are often unaware of their benefits. These benefits must be made known since the acceptance of new technologies is related to consumer perception of the benefits, risks and naturalness of the technology (Siegrist, 2008).

One of the major barriers to the use of pulse proteins as ingredients is their effect on organoleptic properties. The incorporation of high concentrations of pea proteins can significantly affect the texture of some products. With increased substitution of legume protein in bread products, for example, sensory scores of crust and crumb colour decreased (Silaula *et al*., 1989). Flavour is a big issue when incorporating novel food ingredients into products (Karleskind *et al.*, 2004). Low molecular weight peptides and volatile compounds in legume can contribute to the beany, bitter or astringent characteristics that are often associated with these proteins. Although

processing methods such as hydrothermal treatments can reduce these off flavours (McWatters, 1980), additional processing increases the overall cost of the ingredient. The effects of heat and pH on the binding of proteins to flavour components also need to be considered during processing (Heng *et al*., 2004).

Legume proteins have been consumed for many years and are accepted as food ingredients. The authors are not aware of any regulation prohibiting their sale as food ingredients. While interactions between proteins and hydrocolloids such as gum arabic have been shown to improve some functional properties, research done in this area has considered the protein and the hydrocolloid as separate ingredients, rather than a complex that can be marketed as such. Regulations regarding the inclusion of the specific hydrocolloid would have to be factored in to food formulations.

Concerns about allergenicity should not be overlooked. While the legume proteins have not been identified as major allergens, as is the case with soybean, allergenic responses to these proteins have been reported in Europe, Asia and the Mediterranean and there is evidence of some cross-reactivity between these proteins (Boye *et al.*, 2010b). The allergens are heat stable but the immune response can be reduced by hydrolysis of the proteins.

Growing legumes has environmental benefits based on their ability to fix nitrogen. Excessive processing to produce legume protein products increases energy requirements that may counteract some of the benefits associated with the environmental benefits of growing pulses. This is especially true for protein produced through wet fractionation methods (Aguilera *et al*., 1982). To be more environmentally conscious, alternative processing methods should be explored.

Another option that may lead to increased utilization of legume proteins is the production of bioactive peptides by controlled hydrolysis of the protein. Wu *et al*. (2006) have a patent application for the production of peptides from a range of plant proteins including legumes that can inhibit angiotensin converting enzyme (ACE), and aid in the control of hypertension. More work is required related to the efficacy, economics and regulatory issues associated with this use of legume protein hydrolysates.

9.7 References

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10

Wheat gluten: production, properties and application

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Abstract: Gluten, the dough-forming protein of wheat flour, is important for a range of technological applications from supporting the baking performances of leavened products to the development of new food protein ingredients and other biomaterials. The past five decades have seen the rise of gluten as a commodity in its own right, through the large-scale industrial separation of gluten from wheat starch, together with the controlled drying processes to retain its functional properties. The resulting vital gluten is most widely used in bakery products. However, new technologies are being increasingly explored to modify the structure and thus the functionality of this unique protein ingredient to provide a range of functional properties at a more modest price than its competitors such as milk and soy proteins.

Key words: gluten, structure, production, viscoelasticity, modification, emulsifier, food uses, allergy, coeliac disease.

10.1 Introduction

Gluten may be defined as the cohesive, viscoelastic proteinaceous material prepared as a by-product of the isolation of starch from wheat flour. A biological definition might include the origins of the gluten-protein complex as being derived from the 'storage proteins of the wheat grain' (Schofield and Booth, 1983). Gluten, the dough-forming protein of wheat flour, is the key to the unique ability of wheat to produce leavened products.

Although the knowledge of how to fractionate wheat flour into starch and gluten has been known for almost 300 years, gluten has only been traded as a commodity during the second half of the 20th century. The past five decades have seen the rise of gluten as a unique vegetable protein of considerable commercial significance, both as an additive to fortify flour for bread manufacture and as an ingredient for many food and non-food uses. In its most familiar form, gluten is sold in the dried state as 'vital wheat

gluten', which is produced industrially from wheat flour by various wet separation and controlled drying processes. In this form, vital wheat gluten largely retains the original functional properties of wheat gluten upon rehydration.

Its ability to form an elastic mass when hydrated, its water-holding capacity and thermosetting properties allow it to be widely used in a variety of food and non-food applications, some outside the traditional wheat and cereal-based foods. In addition, many modifications to gluten have been developed and are gaining wider application in a range of products. Gluten (vital, de-vital or modified) is finding increasing use as a food ingredient that provides a range of functional properties at a more modest price than its competitors, such as milk and soy-derived protein ingredients.

The term gluten is also used to refer to the protein residue remaining after the isolation of starch from corn (maize). However, this 'corn gluten' is functionally very different from wheat gluten. Another connotation of the term 'gluten' relates to the family of proteins that cause dietary problems for people with coeliac disease. In this case, the term 'gluten' includes the storage proteins from the grains of rye, triticale, barley and possibly oats. In the context of this chapter, gluten is the fraction isolated from wheat flour that is enriched in the major wheat proteins, i.e. gliadins and glutenins. Its industrial production, functional properties and applications as a high-protein ingredient for food, feed and other uses are discussed.

10.2 World production and trade

Wheat gluten is commercially marketed as a cream coloured, free flowing powder (Fig. 10.1a). When mixed with water, it forms a cohesive viscoelastic mass (Fig. 10.1b). As a plant source protein, wheat gluten ranks second to soy-based protein in terms of volume, and has enjoyed steady growth in

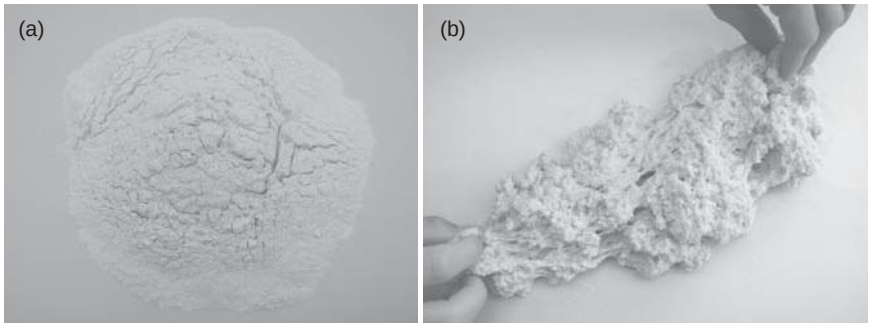


Fig. 10.1 Commercial vital wheat gluten powder (a) and fully rehydrated wheat gluten upon mixing with water (b).

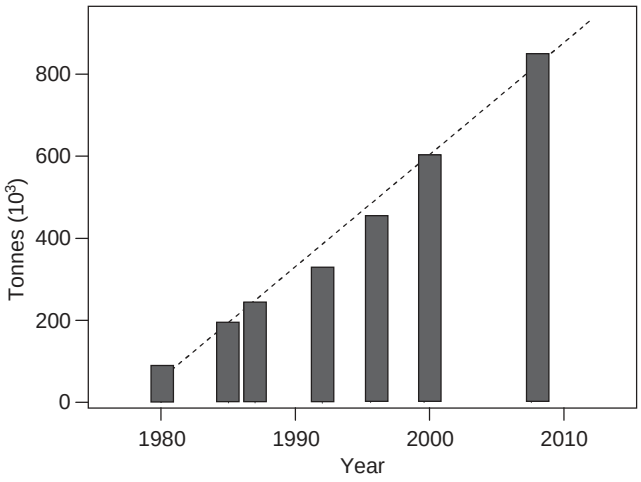


Fig. 10.2 World gluten production between 1980 and 2008 (Krishnakumar and Gordon, 1995).

production worldwide. Its total production has increased from approx. 90,000 tonnes in 1980 to about 850,000 tonnes in 2008, a ~10-fold increase in three decades (Fig. 10.2), with consumption increasing at a similar rate. This production utilizes about 1–2% of annual world wheat production. However, the percentage of wheat used for gluten production varies between the geographic regions and countries. For example, in Australia, about 26% of wheat flour milled is used for gluten production (Dominy, 2005). Australia has been the largest gluten exporter to the United States, but this has gradually decreased in recent years to a rate relative to other geographic origins such as the EU and China (Boland *et al.*, 2005). Almost two-thirds of the 400 million pounds imported to the USA now comes from EU countries, with Australia accounting for 18% of imported gluten in 2006 and China 14% (The US Food and Drug Administration, http://www.fda.gov).

Although gluten production in China has a long history, industrialized production began much later than in Western countries. Enterprises of wheat gluten in China are small in number and size, most of which are small factories with annual production of below 1000 tonnes. These factories are poor in processing control and operate with outdated equipment and low efficiency, thus, the product quality cannot be guaranteed. Overall, gluten production in China is still in its infant stage; however, the introduction of industrialized production of wheat starch/gluten on a large scale in the late 1980s, has resulted in a substantial increase in gluten output domestically. Chinese domestic gluten annual output was reported to reach a total of 200,000 tonnes in 2007, approx. a quarter of the total world production, with an increase at a rate of more than 15% annually since. China has

become a significant gluten exporter, particularly to the United States, due to the low demand of domestic consumption and the low cost of production.

10.3 Wheat gluten manufacturing processes

Gluten was first described and prepared from flour by an Italian named Beccari about 300 years ago (Bailey, 1941). By washing wheat dough in water or diluted salt solution, a cohesive mass "gluten" that contains a high amount of protein, ~75%, is obtained. This discovery, which can be easily reproduced in the home kitchen, has become the basis of a major cereal industry, utilizing millions of tonnes of wheat annually in North America, Europe, Australia and China.

The present commercial process is basically an efficient repetition of Beccari's experiment and involves in principle only a few key steps. Figure 10.3 shows the flow chart of typical processes for the manufacture of gluten and its co-product starch. The first step is mixing the flour with water to form a dough or batter. The dough is allowed to rest and fully hydrated to produce gluten protein agglomerates. The protein fraction is then separated from the starch with additional water, by centrifugation, in hydrocyclones or decanters, or by sieving (screening). The final stages are drying, grinding and sieving to obtain vital wheat gluten in powder form.

Most commercial operations now use variations of either the Batter process or the Martin process (Knight, 1965). In the Martin Process, wheat dough is washed with water while it passes through a tumbling cylindrical

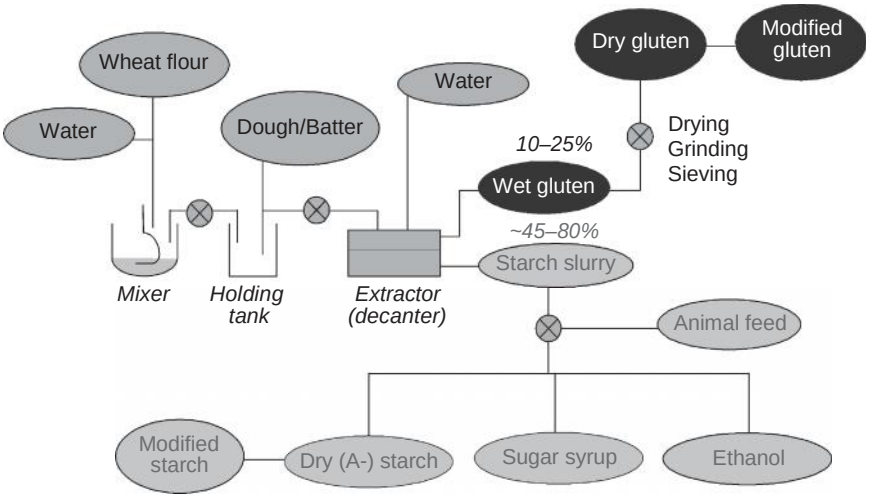


Fig. 10.3 Flow chart of a type of gluten/starch separation and production process.

agitator so that the starch comes out of the dough, while the protein content increases in the remaining dough. The dough is moved along the cylinder by a tumbling action, while the starch passes through small holes in the wall, leaving the protein mass inside to receive further washing until it falls out at the end. On the other hand, the Batter Process involves preparing a thick suspension ('batter') of flour. During several hours of stirring, the starch separates from the gluten, so that when the mixture is passed over a fine sieve, the starch granules separate and the curds of gluten are retained on the screen. Further washing of this gluten removes more starch in a similar manner to the Martin process. Modern applications also use centrifugal techniques (either conventional industrial centrifuges or hydro-cyclones) to separate the starch from the protein. In particular, hydro-cyclones serve at least in cleaning the starch, but also, in some cases, for the actual separation of the starch and gluten. Various process modifications have been made to these basic methods, for example, the Alfa-Laval Raisio process, which is popular in North America and Europe. This process mixes flour with water to produce a homogeneous batter without gluten protein agglomeration. Prime starch is separated first from the protein-rich fraction using continuous centrifugation prior to the separation of gluten with water extractable fraction (Dahlberg, 1978). Detailed engineering aspects and variation of different commercial processes have been described by Grace (1988) and recently by Van Der Borght *et al.* (2005).

The drying stage is critical for retention of functional properties as gluten is very susceptible to heat denaturation when wet, and even relatively low temperatures may destroy gluten's viscoelasticity. Although freeze-drying produces gluten of the highest vitality, it is economically unviable. Most adopted drying methods for commercial gluten use a flash or ring type of drier. The principle of the ring drier involves mixing wet gluten (70% moisture) with dry gluten, thus reducing moisture to about 20%. This material is comminuted and subjected to flash drying. A portion of the dried gluten is drawn off from the drying ring, while new moist gluten is introduced. The rate of drying and the temperature of the moist gluten must be carefully controlled in order to retain the functional properties of gluten.

One critical consideration in gluten washing is the amount of water required per tonne of flour processed, and the disposal of the liquid waste stream, which carries soluble protein, damaged starch, sugars and fibre. Disposal measures include fermentation (generating ethanol or methane), recovery of the suspended and soluble solids and drying the solids for animal feed, and discharge into the sewerage system, but this last option has become less common due to environmental concerns.

10.4 Composition and protein structure

As a commodity, dry gluten must contain a minimum of 80% protein ($N \times 6.25$, or 75%, $N \times 5.7$) as specified by the Codex standard (2001, Table 10.1),

Table 10.1 Codex international standard for wheat protein products including wheat gluten (Codex, 2001)

Protein ($N \times 6.25$) dry basis	>80.0%
Moisture	<10.0%
Ash	<2.0%
Fat (ether extracted)	<2.0%
Fibre	<1.5%

up to 10% moisture, and with the reminder being wheat lipids, residue starch granules and insoluble fibre, which are trapped in the protein matrix and become more difficult to remove as the protein content increases.

10.4.1 Amino acid composition

Among the 10 essential amino acids required for human health that must be provided through the diet, the only amino acid limited in gluten is lysine, with some of the other essential amino acids being present in considerably higher amounts than the requirements in a single protein source (Table 10.2). From a nutritional value of being able to provide balanced essential amino acids similar to other plant source proteins (rice and maize), gluten (or wheat proteins) is considered to be poorer quality than proteins from animal sources. However, gluten protein does contain high levels of the non-essential amino acid glutamine, which serves as an efficient utilizable source of α-amino nitrogen, to meet the demand for the synthesis of non-essential
amino acids in the human body, as well as for the synthesis of other physiologically important nitrogen-containing compounds, which are essential for strengthening and repairing the body muscle (WHO/FAO/UNU, 2007).

10.4.2 Protein structure

Gluten protein represents ~85% of the wheat proteins and is made up of a complex mixture of proteins, containing many, probably several hundred, polypeptides of different molecular weights (Fig. 10.4). About half of the proteins are monomeric gliadins, with the remainder being disulfide cross-linked polypeptides that form the polymeric glutenin fraction, whose size can range up to tens of millions of Daltons (Shewry *et al.*, 2002; Wrigley, 1996). Together, they provide gluten with its unique physical properties – gliadin for viscosity and extensibility and glutenin for elasticity. The appropriate balance of the two is important for baking.

The gliadin fraction contains mainly single polypeptide chains of molecular weight (M_r) in the range of 30,000–75,000 Da. The gliadins associate with each other and with glutenin proteins through non-covalent hydrogen bonds and hydrophobic interactions. The ω-gliadin contains a large

Table 10.2 Amino acid composition of gluten protein, and in comparison to the essential amino acid requirements recommended for adult (mg/g protein)

Amino acid protein	Gluten[a]	FAO/WHO/UNU recommendation[b]
Histidine	21	15
Isoleucine	38	30
Leucine	67	59
Lysine	16	45
Methionine + cysteine	39	22
Methionine	*14*	*16*
Cysteine	*25*	*6*
Phenylalanine + tyrosine	83	38
Threonine	25	23
Tryptophan	11	6
Valine	39	39
Total indispensable amino acids	**339**	**277**
Glutamine/glutamic acid	375	
Asparagine/aspartic acid	31	
Alanine	23	
Glycine	28	
Serine	47	
Proline	120	
Arginine	36	
Total	**1000**	

[a] Calculation from the average determination of three commercial vital wheat gluten produced in Australia between 2005 and 2007.
[b] FAO/WHO/UNU (2007).

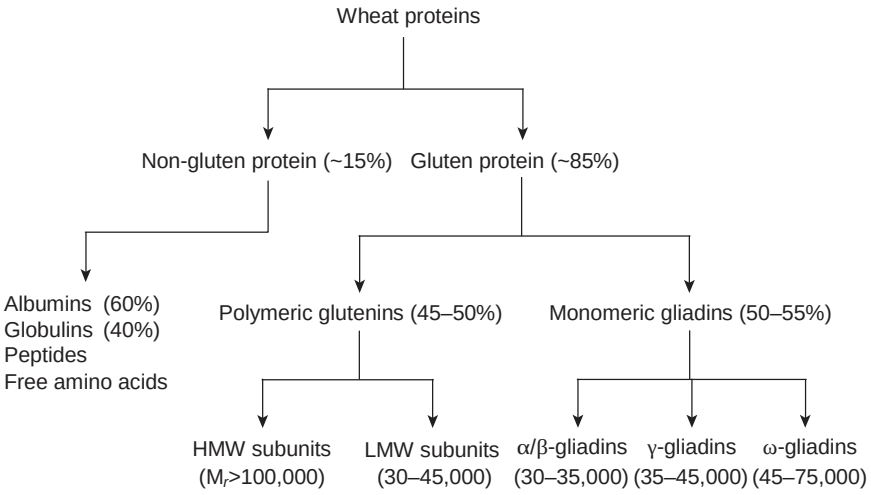


Fig. 10.4 Composition and classification of wheat proteins.

proportion of the amino acids glutamine, proline and phenylalanine; together they account for about 80% of the total residues. They constitute the so-called S-poor prolamins fraction because they have few or no residues of sulphur-containing amino acids cysteine and methionine (Shewry, *et al.*, 1986). In contrast, α -, β - and ɣ -gliadins have less proline, glutamine and phenylalanine, but 2–3 mol.% cysteine plus methionine. The cysteine residues are located towards C-terminal ends and are involved in intramo- lecular disulphide bonds (Shewry and Tatham, 1997). The α /β - and ɣ -gliadins are structurally related with their M_r ranging from 30,000 to 45,000 Da.

The polymeric glutenin proteins are divided into high molecular weight (HMW) and low molecular weight (LMW) subunits. The HMW subunits account for about ~12% of the total gluten protein. Their size (M_r > 100,000 Da) and their ability to form an intermolecular network give the gluten framework its structure. Thus, it is the HMW subunits that are largely responsible for providing gluten viscoelastic properties (Shewry, *et al.*, 2002). The remaining LMW glutenins are polymeric proteins that have amino acid compositions and structure similar to the α /β - and ɣ - gliadins, with a slightly higher M_r of ~45,000 Da. But their ability to form intermo- lecular disulphide bonds with each other and/or with HMW glutenins, is important for the formation of the glutenin macropolymer.

10.4.3 Minor component – wheat lipids

Much of the lipid content of the flour becomes associated with the gluten protein during the washing process. The gluten proteins are largely hydrophobic in nature and the lipids bind to the hydrophobic areas of the protein as they are repelled by the water used in the washing. Therefore, the lipids are strongly bound to gluten proteins and are removed with much more difficulty than they are removed from the original flour. The lipid content of gluten is primarily determined by the lipid content of the flour from which the gluten is produced, and is unaffected by additional washing. Although it is a minor component (<2% free fat, but as high as ~8% bound lipids), the lipid in gluten can affect its functional properties, flavour and quality (e.g., shortened storage stability due to lipid oxidation). One way to improve gluten quality with having a lower lipid content is to use salt during the dough mixing and washing process (Day *et al.*, 2009a). Figure 10.5 shows that the viscoelastic properties of the salt-washed gluten could be enhanced with either the use of 2% NaCl or 0.5% NH_4Cl with the reduction of lipid content to approximately half of the amount in the gluten produced by using water only.

10.5 Functional and sensory properties

10.5.1 Solubility and water holding capacity

The ability to obtain gluten in a relatively pure form with its functionality retained by such a simple process is due to gluten's unique properties.

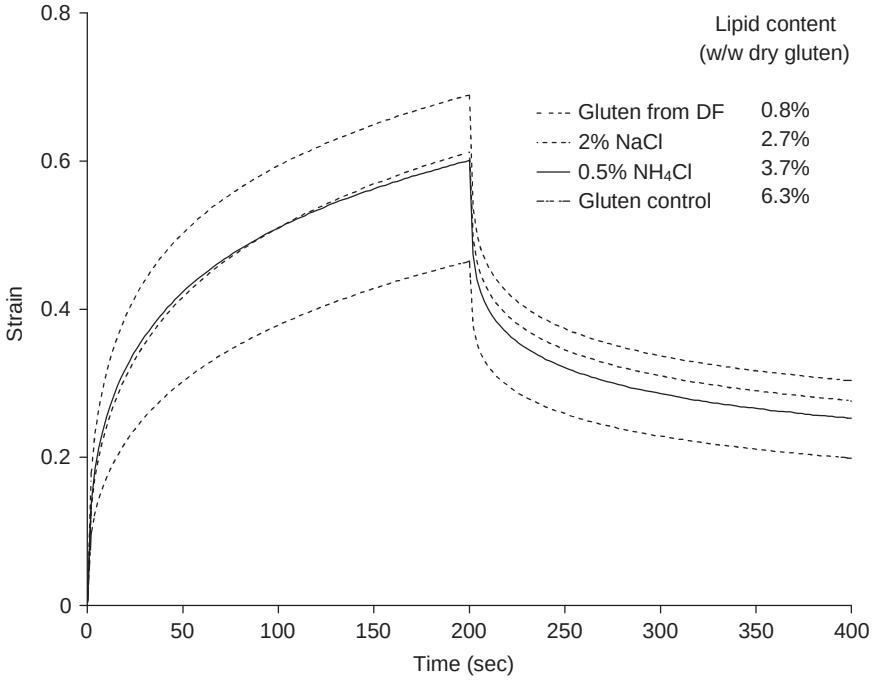


Fig. 10.5 Rheological properties of gluten influenced by salt during processing.

Gluten proteins are insoluble in water and are associated by strong covalent and non-covalent bonds which allow the whole fraction to form a cohesive viscoelastic mass (Fig. 10.1b). Gliadin can be solubilized in 70% aqueous ethanol, one of the steps of the Osborne fractionation of wheat proteins (Osborne, 1924), and the residue after this extraction is glutenin. Gliadin and most of the glutenin may be solubilized to a certain degree under acidic conditions.

When mixed with water, gluten proteins are rehydrated and form a strong rubber-like gel. Vital gluten can take up 1.5 to 2 times its own weight in water. Ionic strength and pH have little effect on the water uptake of vital gluten. The ability of gluten to absorb water rapidly and its capacity to hold water results in increased yield and extended shelf-life for the food systems into which it is incorporated.

10.5.2 Viscoelasticity

Its rheological properties are the basis of the functional uses of vital gluten, and set it apart from all other commercially available vegetable proteins. It is these properties that permit breads, cakes, biscuits and noodles to be

made from wheat-flour doughs. In the wet state, the protein molecules form a cohesive matrix. This matrix is elastic, allowing it to stretch and expand. In aerated doughs, this elasticity permits the expansion of the gas bubbles that produce the texture of bread and cakes. If the gluten matrix in the original flour is too weak, or the protein content is too low to form an effective matrix, the bubbles expand beyond the elastic limit and burst, reducing the overall volume of a baked product. In such cases, fortification with added gluten is essential for the satisfactory production of bakery goods. The viscoelastic behaviour of hydrated wheat gluten is largely due to its low content of charged amino acid residue. Protein molecules are able to associate closely together and resist dispersion.

10.5.3 Flavour

Vital gluten exhibits a flavour note described as "bland" or "slight wheaty". Wheat flavour enjoys wide acceptance and wheat gluten blends perfectly well into all cereal-based products. Blending of wheat gluten with other food proteins which do possess a characteristic flavour can result in improved total flavour. For example, soy/wheat gluten blends are used for textured vegetable protein. In addition, the blending with soy, which is high in lysine content, in an appropriate ratio, can synergistically improve the nutritional value compared to that of either protein alone.

Wheat gluten can suffer from off-flavours, particularly rancidity, caused by lipid oxidation, if it is not properly produced and stored. Thus, having low lipid content in gluten is also important for reducing wheaty or off-flavour notes, particularly for non-cereal based applications.

10.6 Modification of gluten for new functional properties

Due to its low water solubility, vital gluten has poor foaming and emulsifying properties. Deamidation, is one of the approaches that can be used to enhance gluten's solubility. This may be achieved with either acid or alkali treatment (Batey and Gras, 1983). Removal of the amide group from the glutamine residues (to form the corresponding carboxylic acid, glutamic acid) changes the structural conformation of the protein and increases its surface charge, thus increasing solubility at neutral pH (Fig. 10.6). Deamidated gluten is easily dispersible, making it suitable for use in foods for emulsification or for foam stabilization. Due to its unique conserved structure upon adsorption to the oil/water interface, deamidated gluten provides emulsions with a superior interfacial stability against droplet coalescence and heating (Day *et al.*, 2009b). Deamidated gluten can be used in various ways, e.g. to provide water-binding and emulsifying properties for meat products, to provide nutritional advantages to sports drinks and medical supplements, to mimic dairy protein functions in products such as coffee

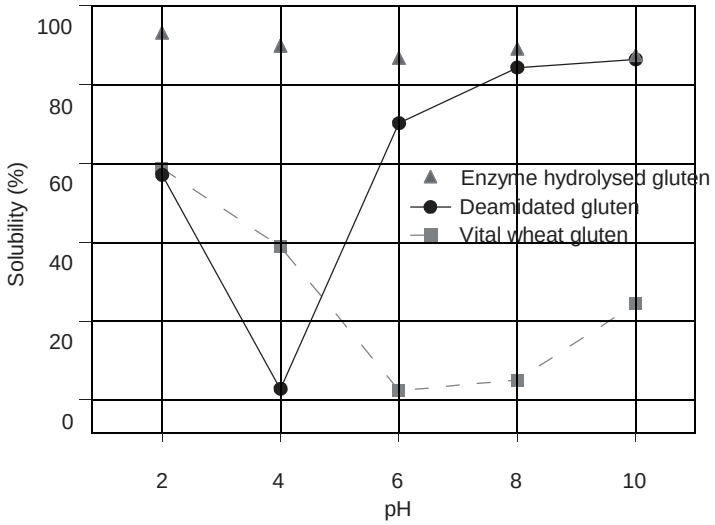


Fig. 10.6 Comparison of the solubility of vital wheat gluten, chemically and enzymically modified gluten at different pH.

whitener and calf milk, as an emulsifier for powdered shortenings, and as milk replacement in bakery applications. Due to the loss of hydrogen bonding interactions within and between the protein molecules, and thus the loss of its viscoelastic properties, deamidated gluten has no benefits for use in bread doughs for functional properties except for the purpose of having high protein content.

The solubility of gluten may also be enhanced by enzymic hydrolysis of the peptide bonds, thereby reducing the sizes of the polypeptide chains. Suitable commercial enzymes include papain, bromelain, subtilisin, trypsin, pronase, and some relatively new proteases such as alcalase, protamex and neutrase, etc. These enzymically solubilized preparations of gluten have many of the properties of chemically deamidated gluten, such as foam stability and emulsion formation. However, unlike chemically deamidated gluten, enzyme-solubilized gluten has beneficial effects on dough properties because the peptides can form covalent and non-covalent interactions with native gluten in flour. For example, the addition of enzyme-solubilized gluten at levels of 1–2% can reduce dough-mixing times by amounts similar to those achieved by the addition of chemicals such as cysteine and ascorbic acid, which are often used commercially to improve loaf volume (Asp *et al.*, 1986). However, enzyme solubilization may lead to products with a bitter taste, due to the release of small peptides by enzyme action, even though the fraction of these small peptides is generally low when gluten is hydrolysed enzymatically.

A third approach to gluten protein modification is the use of physical means, such as heat treatment, extrusion texturization and high-pressure processing, etc. Extrusion technology is used widely to produce a fibrous structure in gluten to simulate meat fibres. Alignment of wheat protein molecules during the extrusion process allows them to form thin filaments or microfibrils that assemble a macroscopic fibrous structure. Hydration of the fibrous strands gives the laminated, fleshy appearance of texturized wheat gluten (Maningat *et al.*, 1999). High pressure has been found to change gluten to either more liquid-like at relatively low pressure (200 MPa), or more solid-like as the pressure was increased to 800 MPa (Apichartsrangkoon *et al.*, 1999). There was evidence of the weakening of non-covalent bonds in mild treatment conditions, but further chemical crosslinks occurred with increasing severity of treatment.

10.7 Uses and applications of wheat gluten

The demand for plant source proteins with special functional properties is growing rapidly in line with the increasing consumption of fabricated foods. The high protein content, unique viscoelastic characteristics, thermosetting and water absorption properties of wheat gluten offer opportunities to food scientists and technologists for innovative product formulation. Vital wheat gluten's unique viscoelastic properties improve dough strength, mixing tolerance and handling properties. Its film-forming ability provides gas retention and controlled expansion for improved volume, uniformity and texture; its thermosetting properties contribute to structural rigidity and bite characteristics; and its water absorption capacity improves product yield, softness and shelf-life.

The most common uses of wheat gluten are in the milling, baking, cereals, meat, pet food and commercial feed industries (Table 10.3). However, with

Table 10.3 Utilization of vital wheat gluten in different regions of the world (as approximate percentage of total usage for the region) and total in the world

Uses	North America	Europe	Australia	Japan	Total world
Milling and flour fortification	2	41	8	–	**14**
Baking	70	45	54	10	**63**
Noodles	–	–	–	10	**–**
Breakfast cereals	12	2	12	–	**2**
Meats analogues	4	1	9	30*	**5**
Processed meat	1	–	–	30	**4**
Pet food	8	8	5	–	**4**
Other	3	2	3	20	**8**

* Includes gluten used for synthetic fish products.

an increasing awareness of wheat gluten's unique structural and functional properties, it has potential for an expanding diversity of applications.

10.7.1 Flour fortification and bakery products

The major usage of gluten in Western countries has traditionally been, and continues to be, in baked goods of various types. In these cases, the gluten is used, by flour millers and bakers, to fortify flours of lower-than-desirable protein content in order to supplement lower protein local wheats and to improve the quality of the flour to equal those with higher protein content. This fortification may be necessary either because the flour is naturally low in protein, and higher protein content is needed to make quality products, or because the addition of gluten provides a particular property sought in the food and achieved by the quality of the protein. Blending dried vital wheat gluten with flour has become increasingly common in parts of Europe (Table 10.3), where gluten fortification of low-protein bread flours offers an attractive alternative to blending with expensive, imported high-protein wheats to satisfy functional performance requirements (Spooner, 1995). Bakers also use gluten to fortify their basic flours at different levels to obtain desired performance for the production of specialty breads and different types of bakery goods. This minimizes flour inventories and avoids storage of high-protein flours.

The level of gluten used can be quite specific, depending on the particular application and the required texture and shelf-life needed for each particular bakery product. For example, addition of about 1% gluten to flour reduces pretzel breakage in the finished product, but the addition of too much gluten may result in pretzels that are too hard to eat. Gluten is used at approximately 2% in pre-sliced hamburger and hot-dog buns to improve the strength of the hinge and provide desirable crust characteristics when buns are stored in a steamer. Gluten can also be used to strengthen pizza crust, making it possible to produce both thin and thick crusts from the same flour. The incorporation of gluten provides crust body and chewiness and reduces moisture transfer from the sauce to the crust.

10.7.2 Breakfast cereals

One of the earliest uses of heat-dried gluten was to produce the breakfast cereal 'Special K' by Kelloggs (Thompson and Raymer, 1958). Wheat gluten provides not only the protein desirable for nutritional claims but also helps to bind vitamin and mineral enrichment components to the cereal or grain in process and contributes to the textural strength of flake cereals. Wheat gluten-fortified breakfast cereals have been widely accepted by consumers because they are very flavourful, nutritious and crispy textured, especially when consumed with milk. Kellogg's Special K and NutriGrain cereals are perhaps the most familiar examples in this product category.

10.7.3 Pasta and noodles

Similar to bakery products, gluten can be added to flours of lower proteins for the production of pasta and noodles. Although durum wheat is preferred for pasta, because of its high protein content and color, other more available flour can be used effectively if wheat gluten is added. Gluten addition can reduce cooking loss and stickiness in cooked pasta and noodles, provide good cooked firmness, increase resistance to breakage, and improve heat tolerance in canned retorted products.

High-protein pasta formulations using vital wheat gluten in combination with soy protein and lactalbumin have been developed; they satisfy the protein quality requirements of the US Type A National School Lunch Program (www.iwga.net).

Japanese noodles such as udon-type are normally made from high-gluten wheat flour similar to bread flour. Gluten addition is required for some commercial production of the pre-cooked white salted noodles that are formulated with the addition of starch (e.g. tapioca starch) to enhance the desired textural properties without cooked noodles becoming soggy and too soft during storage. The added gluten compensates for the decrease in protein content with starch incorporation and improves the dough sheeting properties as well as cooking and textural properties of the prepared noodles.

10.7.4 Processed meat, poultry and fish products

A desired property of gluten is its ability to bind fat and water while at the same time increasing the protein content; together with its film-forming and thermosetting properties, gluten is attractive for various types of applications in meat, fish, and poultry products. Gluten improves the utilization of beef, pork and lamb meats by a restructuring process, which converts less desirable fresh meat cuts into more palatable steak-type products. Gluten has also proven to be a satisfactory binder for turkey-meat pieces because of its ability to produce intact loaves with good slicing qualities. In processed meat products, gluten is an excellent binder in poultry rolls, canned 'integral' hams, and other non-specific loaf-type products, where it also improves slicing characteristics and minimizes cooking losses during processing. Vital wheat gluten is also useful in extending ground meat patties and as a protein binder in sausages and other meat emulsion products.

10.7.5 Texturized vegetarian foods, meat and cheese analogues

A major use of gluten in non-bakery foods is as a meat replacement in vegetarian foods, and in the production of analogues of expensive foods such as seafood and crab meat, particularly in Japan (Table 10.3). Due to the growing concern for health and food safety, an increasing number of consumers are looking for meatless alternatives. Pure wet wheat gluten can

be seasoned, shaped, and cooked into meatballs and steaks. Texturized wheat gluten developed using extrusion technology can be used to mimic the mouthfeel, chewiness, and taste of meat (Maningat *et al.*, 1999). Meat substitute products created by this process are suited to ready-to-eat entrees, as sandwich fillings, or for pizza and salad toppings. Gluten also acts as a binder and provides a meat-like structure in 'veggie burgers'.

Gluten's viscoelastic properties can be used in preparing synthetic cheese with the characteristic texture and eating quality of natural cheese. Gluten alone or in combination with soy protein, has been used to replace approximately 30% of the more expensive sodium caseinate used in imitation cheese products.

10.7.6 Traditional Asian /Chinese food

Perhaps one of the most popular uses of wheat gluten is in Japan and China, where it was first developed, as well as in the cuisines of other east and southeast Asian nations. It is believed that wheat gluten originated in ancient China, as a meat substitute for adherents of Buddhism. In traditional Chinese cuisine *Miàn jīn*, the wheat gluten ball is deep fried before being cooked in, which confers a crispy rind that enhances the texture of the gluten. Simulated duck is another common example of wheat gluten use. Wheat gluten is also an alternative to soybean-based meat substitutes such as tofu.

Gluten is also used to prepare soy-sauce extenders, and to manufacture mono-sodium glutamate. The high glutamine content of gluten makes it an ideal starting material for this latter product. Soy sauce made using gluten has light colour, slow browning rate, excellent flavour and good body over traditional soy sauce.

10.7.7 Pet foods, aquaculture and animal feeds

The pet food industry is the second largest user of wheat gluten. The most common application of gluten in pet food is as a binder for re-formed meats and meat pieces, where its water absorption and fat-binding properties can improve yields and quality. The meat chunks are made from emulsified meats in which a small amount of wheat gluten is added to help bind the meat during the cooking process. This gives the meat chunks strength to retain their shape during processing and retorting. Gluten not only binds chunks of raw and cooked meat together, but it also absorbs the natural juices of meat that would otherwise be lost during the cooking process.

Another increasing use of gluten is in aquaculture feed. The protein and lipid-rich feed pellets used for farmed fish and shrimp have traditionally been made in part from small, bony fish species that are not generally used for human consumption. However, the relatively high cost of fish meal and fish oil and growing pressure to reduce dependence on marine fish resources

have made feed manufacturers and seafood farmers search for alternative feeds. Gluten's adhesive properties provide the binding needed for the pellet or granule forms of feed commonly used. Its water insolubility reduces pellet breakdown and its viscoelastic properties can provide a chewy texture that is preferable to extremely hard pellets. It also provides nutritional value at a low cost.

Gluten and modified gluten have also been used in calf-milk replacements. Because of the variability in price of skimmed milk powder, some vegetable proteins are being considered as an alternative source of protein to provide the amino acids required by young calves. Gluten has been used in fat-filled powders to feed piglets (e.g., Amytex and Solpro from Tate & Lyle).

10.7.8 Non-food uses

Apart from use as a high protein food ingredient, gluten has also enjoyed wide use in non-food applications (Bietz and Lookhart, 1996). Many of these applications, such as films, plastics and adhesives, relate directly to gluten's adhesive, cohesive and elastic properties.

Gluten has the ability to provide edible films that protect food or food components from interactions with the environment as they can serve as a barrier to mass transfer (e.g., oxygen, water vapour, moisture, aroma, lipids). Gluten-based films may be casted from solutions of gluten in ammonia or alcohol. The properties of wheat gluten film may be altered by the pH, or solvent concentration of the film-forming solution, or by heat treatment. As there can be processing issues such as altered rheology and flow properties, especially under conditions of heat, this requires an understanding and matching of the relative importance of the various functionalities of films, such as thermosetting, moisture and oxygen barrier properties, cohesiveness and durability to its intended application (Guilbert *et al.*, 2002).

Production of gluten films with satisfactory properties may provide new biodegradable films for more widespread uses. Technological approaches to make gluten-based materials using wet processes and dry processes such as extrusion or compression moulding to exploit thermoplastic properties of gluten proteins and potentially produce useful film materials were recently reviewed (Lagrain *et al.*, 2010). In a recent EU study (FAIRCT961979: www.cordis.europa.eu), it was found that the differences in mechanical properties induced by the process of film preparation were larger than those arising from variations of protein composition and properties (except for films cast from water dispersion) due to the wheat genotype, including durum wheat. Therefore there is no need for specific breeding as far as uses of wheat proteins for non-food film materials are concerned. Hence wheat unsuitable for bread- or pasta-making or low-quality gluten could be used for preparing plastic films.

Manufacturers of personal care and cosmetic products are increasingly seeking to use "natural" ingredients of plant origin to replace animal proteins due to pressures from animal rights activists and consumer advocates. Hydrolysed wheat protein is widely used in skin care and hair care formulations such as lotions, creams, shampoos, conditioners, soap bars, toothpaste and shaving products. Gluten hydrolysate promotes effective film formation, moisturizing and foaming properties. Because of the presence of cysteine residues, wheat protein and its hydrolysed peptides can form covalent disulphide bonds with α -keratin proteins in human hair resulting in substantivity or a permanent conditioning effect (Maningat *et al.*, 1994).

There are also a number of other new and potential applications. For example, gluten's adhesive properties make it useful in pressure-sensitive medical bandages and adhesive tapes; its reactivity makes it useful for binding heavy metals in industrial processes, removing ink from waste paper, or solidifying waste oils; and gluten's hydrophobic and (in)solubility has potential for slow-release encapsulation of pest- or weed-control agents (Quimby *et al.*, 1994).

10.8 Regulatory status and gluten intolerance

Vital wheat gluten is approved by the US Food and Drug Administration as Generally Recognized as Safe (GRAS) for use as a dough strengthener, formulation aid, nutrient supplement, processing aid, stabilizer and thickener, surface-finishing agent and texturizing agent at levels not to exceed current good manufacturing practice. Vital wheat gluten also complies fully with the requirements for purity and identity stipulated by the joint FAO/WHO Expert Committee on Food Additives and by a Codex standard (Codex, 2001). Vital wheat gluten is approved for use by most countries throughout the world. However, due to the low protein digestibility corrected amino acid score (PDCAAS) of gluten in comparison to proteins from animal sources and soy, it prohibits protein enrichment claims in the USA for food products containing added gluten.

Although safe to use as a protein ingredient from an industrial practice point of view, like many other food proteins such as those from dairy or soy sources, gluten may provoke an adverse reaction when ingested. Coeliac disease, a well-known permanent food intolerance to wheat gluten (and similar proteins of barley and rye) identified over a century ago, is characterized by inflammation of the small intestine resulting from an inappropriate immune response to wheat gluten (Trier, 1991). This disorder adversely affects the absorption of water and nutrients causing, in some cases, malnutrition. At present, the only treatment is a strict diet avoiding all products containing gluten.

Dietary intolerance of gluten is not an issue when gluten is used or added to wheat grain/flour-based food products, since the protein is likely to be

present in the products already. However, with an increasing use of gluten and modified gluten into a range of foods that do not traditionally contain wheat or cereal proteins, there is a concern about the effects of gluten on people with coeliac disease and those who appear to have wheat-protein intolerance without diagnostic symptoms of coeliac disease.

Researchers have been very active in trying to identify the mechanism of coeliac disease and to develop alternative treatments for those who suffer from the disease. One approach is to use oral enzyme supplements designed to accelerate gastrointestinal degradation of proline-rich gluten, especially its proteolytically stable antigenic peptides. Other strategies aim at interfering with the activation of the gluten-reactive T cells, including the inhibition of intestinal tissue transglutaminase activity and blocking the binding of gluten peptides to the HLA-DQ2/DQ8 molecules (human leukocyte antigen). Medicinal treatments such as cytokine therapy, or selective adhesion molecule inhibitors that could interfere with inflammatory reactions are showing promise in the clinical research for other gastrointestinal diseases and so may be applicable for patients with gluten intolerance and coeliac disease (Sollid, 2000; Sollid and Khosla, 2005).

Although recent advances have improved our understanding of the molecular basis for this disorder and the development of several attractive targets for new treatments, the technological approaches to decreasing gluten intolerance prior to its consumption have largely been slow. A number of potential approaches have focused on enzyme-aided processing in an attempt to reduce the allergenicity and/or toxicity of gluten. The use of acidic oxidative potential water (an electrolysed strong-acid solution containing active oxygen species) has been shown to be able to break down gluten proteins, thereby enhancing digestibility and lowering allergenicity of wheat proteins (Matsumoto, 2002). The allergenicity of wheat flour prolamins can be decreased by treatment with the protease bromelain (Tanabe *et al.*, 1996). The addition of thioredoxin-H reduces intramolecular disulfide bonds in prolamins, before its use in breadmaking, and has shown significant potential in reducing gliadin immunoreactivity in common wheat (Waga *et al.*, 2008). The deamidation process has also been reported to reduce IgE-binding of gluten allergenic protein by modifying the glutamine residues in the epitope.

Scientific knowledge about coeliac disease, including knowledge about the proteins that cause the disorder and the cereal grains that contain these proteins, is still incomplete. Finding a technical solution to reducing the allergenicity of the proteins by modifying the allergen structure in such a way that the allergenic epitopes are no longer recognized by the immune system remains a significant challenge to the industry. Any consideration of new applications of gluten or modified gluten, particularly in non-traditional cereal-based food products, should consider the impact of such application on those populations with dietary intolerance to gluten and related cereal proteins.

10.9 Future trends

Consumers are becoming environmentally conscious and are looking for more healthy lifestyles, including more natural products in terms of foods, cosmetics and packaging. Thus, there are opportunities, but also challenges, for plant source protein ingredients such as gluten, modified gluten and wheat proteins in general, to be adapted for an expanding range of applications.

Unlike milk and soy proteins, gluten or wheat proteins are not high in biological value when evaluated in isolation as sole protein source and have not been widely researched for nutritional advantages, perhaps in mixed diet including other proteins, etc. On the other hand, they may have been disadvantaged slightly by their link with coeliac disease. Extensive research and education of consumers is needed to fully understand the value of gluten protein in terms of its possible or particular nutritional–health benefits or defects.

While the insoluble nature of gluten is a desirable attribute in the traditional applications of this ingredient such as bread and baked products, where it is essential for their structural properties, its insolubility in water limits its usefulness in many other applications. Thus the developments of soy protein as an ingredient should serve as a model for gluten utilization in the ways it has been changed to develop its particular physical functional properties to suit or enhance its performance in a particular food system. Again, in order to fully utilize this low-cost plant protein, the physicochemical properties of gluten need to be understood and tailored to suit its intended purpose.

Gluten is a modestly priced food protein, much cheaper than milk or soy protein products. Its price advantage offers significant scope for value addition by modification. The functional properties of wheat gluten, which other products cannot duplicate, give it a unique place among the various protein products. However, gluten, modified gluten and its fractions need to compete on price and fitness-for-purpose with other protein ingredients, if wheat proteins are to be successful in a wider food market. This can be achieved by understanding customer needs and by further exploring opportunities which may lead to enhanced nutritional and physical functionality as well as the health benefits of wheat proteins.

Modification of gluten by chemical and enzymatic methods has traditionally been used to improve its solubility and to alter its inherent functionality, thus widening the use of gluten. A range of soluble wheat proteins is now available on the market and they are finding applications in foods where the more expensive milk and soy proteins have been used. Fractionation of wheat gluten protein to its constituents, gliadin and glutenin, also has an available market as a new range of wheat protein-based ingredients (MGP Ingredients, Inc.). Gliadin has excellent film-forming properties and this functionality can be exploited in applications where surface properties are

important. Recently a new water-soluble hydrolysed wheat protein has been developed to deliver high levels of glutamine in sports drinks and nutrition bars (Day *et al.*, 2006). These are good examples of how innovation and advances in science and technology have gradually found wider applications for gluten. They will continue to do so.

However, for such applications to succeed, we need continued fundamental studies of gluten and of how its properties can be changed and enhanced through means of chemical, enzymic, and physical modification using conventional, improved and new emerging processing technologies. Many scientific advances have been made to determine the chemical and molecular properties of gluten proteins. These studies should provide avenues for new concepts and new ideas to modify the properties of gluten and offer hopes for discovering new high-value markets so important for the expanded use of wheat by industry. Researchers need to work closely with the industry, first, to extend our basic knowledge; second, to devise the most economical methods for producing gluten or its fractions for the desired functionality; and third, to search for end uses through modification or conversion to new products. Opportunities for future use lie in elucidating and exploiting specific properties through fundamental and exploratory research, and the challenge today is to continue and expand such research necessary to provide basic information that makes all applications possible.

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11

Canola and other oilseed proteins

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Abstract: This chapter discusses the potential for using some of the lesser known oilseed proteins, in particular canola protein, for human consumption. The impact of different methods for recovering oilseed proteins on the specific proteins recovered, undesirable factors eliminated and functional properties are presented. Two distinct canola protein isolates can be recovered and this is the norm for companies working with canola protein. Potential applications focus on the commercial canola protein products, with some reference to other oilseed products including those from flax and hemp. The chapter concludes with some thoughts on the current status and future for commercial canola products.

Key words: oilseed proteins, canola protein, rapeseed protein, flax protein, hemp protein.

11.1 Introduction

There is a wide range of crops grown primarily because of their ability to store lipids in the form of oil. Many of these oilseeds contain a significant amount of protein that remains in the meal following oil removal. Based on February 2010 data on world oilseeds markets (United States Department of Agriculture, 2010) the leading sources of oilseed meal were soybean (58.8%), rapeseed/canola (13.7%), cottonseed (9.2%), sunflower (7.1%) and peanut (7.1%). Production levels are shown in Table 11.1. With the exception of soybean, the use of these proteins as food ingredients remains relatively minor. As soybean proteins are presented in Chapter 8 of this book, this chapter focuses on canola and other oilseed proteins for which the protein is a major component in the meal, particularly those for which commercial protein products are available. Lupines, which are classified as oilseeds and legumes, have been mentioned in Chapter 9.

A summary of oilseeds that represent potential sources of protein for human consumption is provided in Table 11.1, where soybean has been included for comparison. In general, the protein levels in most non-soybean

Table 11.1 Potential sources of oilseed protein

Oilseed – common name	Botanical name	Production level (top 5 in 2008–9) (million metric tons) (USDA, 2010)	Main producer (top 5 in 2008–9) (USDA, 2010)	Percent protein in seed	Reference for protein content	Uses of protein
Canola/ rapeseed	*Brassica napus, rapa, juncea*	58	Canada	17–26	Xu and Diosady, 1994a	Animal feed Source of protein
Cottonseed	*Gossypium* species	41	China	24–26	Betrand *et al*., 2005	Livestock feed
Crambe	*Crambe abyssinica*			20	Massoura *et al*., 1998	Possible for food and feed
Flax	*Linum usitatissimum* L.			27	Oomah and Mazza, 1998	Baked goods, Cattle feed
Hemp	*Cannabis sativa* L.			25	Yin *et al*., 2007	Dietary supplement
Mustard	*Sinapus alba*			20–30	Xu *et al*., 2003	Condiment, spice Ground to flour
Peanut	*Arachis hypogaea* L.	34	China	25–30	Davis *et al*., 2007	Gluten-free flour
Safflower	*Carthamus tinctorius* L.			13–17	Prakash and Narasinga Rao, 1986; Paredes-López, 1991	Bird seed
Sesame	*Sesamum indicum*			18–25	Elleuch *et al*., 2007	Whole or ground seeds in food
Soybean	*Glycine max*	211	United States	37	Lampart-Szczapa, 2001	Food and feed
Sunflower	*Helianthus annuus*	33	Russia	20–40 (dehulled)	González-Pérez and Vereijken, 2007	Snack food Livestock/bird seed

oilseeds (some varieties of sunflower being the exception) are noticeably lower than that of soybean and as a result it is not surprising that utilization of soybean protein is more advanced. The genus *Brassica* includes mustard, rapeseed and canola varieties. The term canola was introduced by Canadian rapeseed breeders in the 1970s to refer to varieties containing low erucic and low glucosinolate content. In Canada, varieties with a low level of erucic acid (<2% in the oil) and glucosinolates (<3 moles aliphatic glucosinolate per g of meal), continue to be referred to as canola while varieties that do not meet these criteria are referred to as rapeseed. This designation is not used worldwide and there are varieties of rapeseed that have low erucic acid and glucosinolates. In this chapter, which is focused on food proteins, only canola and those rapeseed proteins with low levels of glucosinolates will be addressed and the term canola will be used. While there has been research done looking at isolation and utilization of a number of these proteins, canola proteins are more abundant and more work has been done with these proteins. As a result, this chapter will look primarily at the isolation of canola protein, as well as characterization, and functional properties of these concentrated or isolated proteins. Two major proteins have been identified in canola. The larger one is a salt soluble 12S protein also known as cruciferin, and the smaller one, a 2S protein referred to as napin. Where appropriate, information on other oilseed proteins is included.

11.2 Processing and protein isolation

11.2.1 Dehulling

Dehulling has been used as a pre-treatment for a number of oilseeds and there have been attempts to do this with canola as well (McCurdy, 1992; Thakor *et al*., 1995; Kracht *et al*., 2004) but in small seeds, such as canola, separation is difficult as the hull tends to adhere to the endosperm (Thakor *et al*., 1995). It is believed that lower fibre levels will improve the ability to use the resulting meal as animal feed. In dehulling, the seed were dried, deformed in a single-roll dehuller, separated into kernels and hull fractions using an electromagnetic separator and then the oil was extracted from the kernel fraction (Kracht *et al*., 2004). The reduction in fibre content (~39%) resulted in the production of a meal with a 10% improvement in digestibility. This process also reduced phytic acid content in the meal, but the phenolic acid sinapine and glucosinolates actually increased. Pre-treatments using infrared heating (McCurdy, 1992) or soaking and drying the seeds prior to mechanical dehulling (Thakor *et al*., 1995) did improve dehulling efficiencies. With the infrared pre-treatment, however, the amount of meal produced was unacceptably low (McCurdy, 1992). While there appear to be advantages to removing hulls prior to oil extraction, this remains a challenge for the small canola seed and at this point in time, the feasibility of doing so has not been established.

11.2.2 Oil extraction in relation to protein quality

As the primary product from these seeds is oil, oil extractions have traditionally been optimized for oil recovery while providing a meal that can be used for animal feed. This has not always been in the best interest of protein recovery in that the heat treatments (120–140°C) that were used to inactivate enzymes and remove residual hexane have affected protein solubility and the amount and quality of protein that can be obtained during protein isolation. It is not surprising that efforts have been made to address this problem. Milanova *et al*. (2006) used a meal that had been desolventized using air at temperatures below 50°C to improve the yield of protein in their isolate. BioExx® has taken the approach where they are responsible for both oil and protein recovery and have developed a patented method using a refrigerant-based solvent with a boiling temperature of approximately –20°C (BioExx®, 2010). As a result solvent recovery occurs at temperatures lower than 50°C, below the point where proteins denature.

11.2.3 Methods for isolation of protein

In addition to producing a high protein product, isolation of oilseed proteins aids in the removal of chemical compounds, which are beneficial to the plant, but create problems when consumed by humans. For all oil-producing plants, the fibre, protease inhibitors, phytic acid and phenolics can limit their use. For canola, gluconsinolates are of concern, while gossypol in cottonseed and chlorogenic acid in sunflower must be addressed to use these proteins for human consumption.

Isolation of canola protein normally involves extracting the protein from the food matrix and then recovering the protein from the extract. Steps to further reduce the undesirable factors can be incorporated into this process. A summary of the common extraction and recovery conditions reported in the literature is given in Table 11.2.

The idea of recovering the proteins from canola has been around for a long time. Initially, mild extraction conditions (pH 7) with the addition of 0.25 to 2% sodium hexametaphosphate (SHMP) were used (Thompson *et al*., 1982; Liu *et al*., 1982). By extracting the meal twice with 2% SHMP and precipitating by dilution at pH 2.5, a product containing over 77% protein was obtained, although only 53% of the protein was recovered. A decrease in the SHMP concentration to 0.025% improved the protein content of the isolate (89%), but the yield was even lower (Liu *et al*., 1982). Isolate colour was improved by washing with ethyl alcohol or adding sodium metabisulfite during extraction without affecting yield (Liu *et al*., 1982).

In an effort to increase yields, aqueous alkaline solutions were introduced to solubilize canola protein and this method is often reported in the literature (Wilska-Jeszka and Zajac, 1984; Diosady *et al*., 1989; Deng *et al*., 1990; Tzeng *et al*., 1990; Chen and Rohani, 1992; Xu and Diosady, 1994a; Klockeman *et al*., 1997; Aluko and McIntosh, 2001). In general, as the pH

Table 11.2 Isolation conditions for canola protein from canola meal in the scientific literature

Extraction medium	Protein recovery	Fraction used	Protein concentration ( )	References
Sodium hexametaphosphate (0.25–2%)	Dilute at pH 2.5–4.5	Precipitate	77–89	Liu *et al*., 1982; Thompson *et al*., 1982
NaOH, pH 9.5–12	Adjust to pH 3.5–6.5	Precipitate	87–100	Wilska-Jeszka and Zajac, 1984; Deng *et al*., 1990; Tzeng *et al*., 1990[1]; Chen and Rohani, 1992; Xu and Diosady, 1994a; Klockeman *et al*., 1997; Aluko and McIntosh, 2001; Xu and Diosady, 2002[1]
NaOH, pH 9.5–12	Adjust to pH 6.5	Supernatant	91–96	Tzeng *et al*., 1990[1]; Xu and Diosady, 2002[1]
0.1 M NaCl pH 5.5	Dilution in cold water	Precipitate	78	Murray, 2001a; 2001b; Ismond and Welsh, 1992; Léger and Arntfield, 1993
0.1 M NaCl, pH 8.0	85% $(NH_4)_2SO_4$	Precipitate	na	Wu and Muir, 2008

[1] Oil form starting meal removed with a mixture of methanol, ammonia, water and hexane.
na – not available.

of the extraction is increased, the overall yield of protein also increased, but at high pH values (~pH 12) lysinoalanine is also produced and the performance of the isolates is less than ideal (Deng *et al*., 1990). One concern with using high pH extracts was the dark colours and bitter tastes of the resulting isolates, believed to be due to phenolic compounds (Xu and Diosady, 2002). Ultrafiltration/diafiltration of the extract was introduced to address this issue (Xu and Diosady, 2002).

The desire to maximize protein recovery while retaining protein quality has resulted in limiting the level of alkali present during extraction. Using 0.4% NaOH, only lysine was reduced relative to the starting meal, and no lysinoalanine was formed in an isolate that contained 87% protein (Klockeman *et al*., 1997). Evaluation of the nutritional quality of this isolate indicated that it was equivalent to soybean for 10–12 year olds and adults, but inferior to soybean for infants and 2–5 year olds. According to Klockeman *et al*. (1997), poor solubility between pH 5 and 7 was a concern in terms of effectively using this isolate as a food ingredient. Mild alkaline conditions (pH 8.0) were also used by Raab and Schwenke (1984) and Wu and Muir (2008) to maintain protein structure prior to isolating individual proteins.

An alternative approach to extract canola protein is to avoid alkaline conditions. As many storage proteins are globulins, salts such as NaCl can be used to extract canola proteins. Unfortunately this results in a decrease in protein yield as canola proteins are less soluble in salt solutions than alkali (Klockeman *et al*., 1997). While 91% of the protein dissolves in 0.4% NaOH, only 51% dissolved in NaCl. Some researchers have accepted this lower yield to avoid the structural changes in the protein due to pH extremes and thereby producing a better quality ingredient (Ismond and Welsh, 1992; Léger and Arntfield, 1993; Murray, 2001a; 2001b; Barker *et al*., 2002; Ser *et al*., 2008; Wu and Muir, 2008). Salt levels of 0.1 M (Ismond and Welsh, 1992; Léger and Arntfield, 1993), 0.15 M (Cameron and Myers, 1983; Milanova *et al*., 2006) and 0.5 M (Ser *et al*., 2008) are often used. Another option is to extract with water only (Gosnell *et al*., 2007). A water extraction was used by BioExx® for one of their protein isolates. Ultrafiltration of the salt extracts was included to reduce the undesirable components and concentrate the protein prior to recovery. Based on differential scanning calorimetry (DSC) data, the protein's thermal properties indicated that the salt extract, when coupled with mild protein recovery maintained the protein in its native state (Ismond and Welsh, 1992).

Protein recovery originally focused on ways to precipitate the extracted protein, such that undesirable components would remain in the supernatant. The proteins that were extracted using SHMP were precipitated by dilution in acidic water (Liu *et al*., 1982; Thompson *et al*., 1982). Following washing and neutralization, an isolate with 77% protein was obtained. For alkaline extracts, protein precipitation has been by adjustment of the pH to a more acidic environment, using pH values from 3.5 (Klockeman *et al*., 1997) to 6.5 (Tzeng *et al*. 1990; Xu and Diosady, 1994a), producing isolates

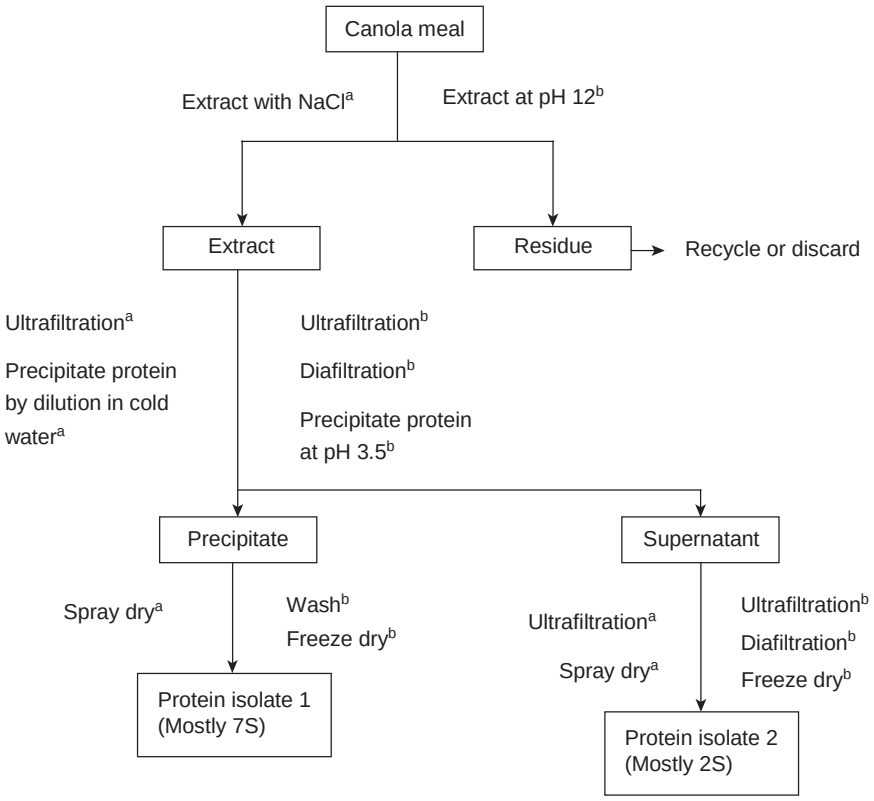
with 87% (Tzeng *et al*. 1990; Klockeman *et al*., 1997) to 99% protein (Xu and Diosady, 1994a).

Precipitation of the protein from the salt extracts was generally done by reducing the ionic strength of the extract; this was done by diluting the extract in cold water (Cameron and Myers, 1983; Ismond and Welsh, 1992; Léger and Arntfield, 1993; Logie and Milanova, 2004; Ser *et al*., 2008). The ratio of extract to water varied. Using 0.1 M NaCl for the extraction, Léger and Arntfield (1993) used a 1:6 extract to water ratio while Ser *et al*. (2008) used a dilution ratio of 1:15 with a 0.5 M NaCl extract. Other options to precipitate what is essentially the 7S canola protein from salt extracts have included isoelectric precipitation at pH 3.5 (Segall *et al*., 2007) and a heat treatment (Gosnell *et al*., 2007).

Low yield in many of these systems has resulted because the lower molecular weight 2S protein did not precipitate. As a result, this protein has been recovered as a second fraction following removal of undesirable components from the supernatant using ultrafiltration and diafiltration as shown in Fig. 11.1 for generalized schemes for two patented processes (Cameron and Myers, 1983; Diosady *et al*., 2005) and seen in an array of patents and patent applications from Burcon NutraScience® (including Logie and Milanova, 2004; Milanova *et al*., 2006; Segall *et al*., 2007). While there is some cross-contamination between fractions, the precipitated protein is predominantly 7S and the protein from the supernatant is predominately 2S. For Burcon NutraScience®, these correspond to Puratein® and Supertein™, respectively, although these terms have not been used in all the patents. By recovering two fractions, the overall protein yield is increased. Tzeng *et al*. (1990) recovered 43% of the canola protein in the precipitated fraction and a further 33% from the supernatant for a total yield of 76%. The process used by BioExx® also recovers both proteins but the Vitalexx™ is from the initial water extract and the Isolexx™ is a product of hydrolysis of the material that was not extracted.

11.2.4 Changes in antinutritional factors during protein isolation

In general, antinutritional factors are reduced due to protein isolation. Glucosinolates were reduced to an undetectable level and 80% of the phytates were removed when SHMP extracts were precipitated at pH 2.5 (Liu *et al*., 1982). However residual phosphorous levels limited the amount of the isolate in feed rations (for rats) to 20%. The inclusion of ultrafiltration and diafiltration in a method where precipitated and soluble proteins were recovered from alkaline extracts of a meal produced by using a $CH_3OH/NH_3/H_2O$-hexane also eliminated glucosinolates and reduced the phytate by 90% (Tzeng *et al*., 1990) to 97% (Xu and Diosady, 1994a). In these studies the phytate concentration was further reduced by the inclusion of $CaCl_2$ during the protein precipitation. Phenolic acids were decreased by 17 and 22% and condensed tannins by 32 and 24%, in the isoelectric isolate



[a] Adapted from Cameron and Myers, 1983

[b]Adapted from Diosady *et al*., 2005

Fig. 11.1 Generalized schematic diagram for isolation of canola proteins (7S and 2S) in two fractions.

and soluble isolates, respectively, by the inclusion of ultrafiltration and diafiltration of the extract and soluble fraction (Xu and Diosady, 2002). When NaCl and SDS were included during the ultrafiltration of the extract, the removal of phenolics and condensed tannins was increased to 70% and 90–95%, respectively, and were further reduced in the soluble fraction by the inclusion of polyvinyldichloride (Xu and Diosady, 2002). An added benefit to these reductions was the lighter colour and blander taste for the resulting isolates.

Using ultrafiltration of the salt extracted protein, Ismond and Welsh (1992) removed 75% of the phytic acid, 85% of the phenolic compounds and 92% of the glucosinolates. In a subsequent study Ser *et al*. (2008) demonstrated that the ultrafiltration step was critical to glucosinolate reduction,

although there were some losses during the precipitation step (for the precipitated protein). It was also noted that the reduction in aliphatic gluconsinolates (~85%) was higher than the reduction in aromatic glucosinolates (36–66%).

11.3 Characterization of canola and other oilseed proteins and isolates

11.3.1 Isolate composition

When looking at the composition of protein isolates, the protein concentration is of importance. As can be seen in Table 11.2, protein concentrations in isolates prepared from canola range from 77 to 100% (using 6.25 as the nitrogen to protein conversion factor). When looking at the literature on protein concentrations in commercial material, the protein levels for the Can Pro SP and Can Pro IP (MCN, 2010) are listed as 60 and 68% (Table 11.3). As the methods to produce these protein products are not available, it is possible that efforts have been made to concentrate the protein, but the proteins have not been isolated. For the isolates from BioExx® and Burcon NutraScience®, the reported protein contents are above 90% and fit the accepted definition of a protein isolate.

One of the features that makes the canola protein attractive for use in human foods is its well balanced amino acid composition. In a comparison of amino acid data for commercially prepared protein products, the levels of indispensible amino acids surpass the WHO/FAO/UNU (2007) requirements for all amino acids, with the exception of Puratein®, from Burcon NutraScience® which is deficient in lysine. Otherwise the canola materials have the lysine that tends to be low in cereals and the sulphur amino acids that tend to be low in legume proteins. Overall, the data support the idea that canola provides a well balanced amino acid profile.

11.3.2 Structure of recovered protein

The major protein components in canola isolates have been reported to be the 12S (cruciferin) and 2S (napin) proteins. The 2S protein is a water soluble albumin composed of two polypeptide chains (9.5 kDa and 4 kDa) joined together by two disulfide bridges (Monslave *et al.*, 1991). As this protein is water soluble, it remains in solution during the precipitation step in most isolation protocols, and is generally recovered from the supernatant as a soluble protein. The 12S protein has a molecular weight of ~300 000 kDa and accounts for 60% of the seed protein (Schwenke and Linow, 1982). However the 12S protein can dissociate to a 7S form and ultimately a 2S form depending on ionic strength, pH and the presence of denaturing agents such as urea (Schwenke *et al.*, 1983). While the 12S protein can be present, the protein in precipitated protein isolates seems to be the form of

Table 11.3 Protein and amino acid composition of commercial canola protein isolates

Property	WHO/FAO/UNU (2007) indispensable amino acid requirements	MCN™ Can Pro SP	MCN™ Can Pro IP	Burcon NutraScience® Supertein™[b]	Burcon NutraScience® Puratein®[b]	BioExx® Vitalexx™[c]	BioExx® Isolexx™[c]
% protein (dry basis)		60	68	>90	>90	87	91
Histidine[1,2]	1.5	4.0	2.9	2.9	1.6	3.0	2.7
Isoleucine[1,2]	3.0	3.6	4.7	3.2	5.1	4.8	4.3
Leucine[1,2]	5.9	6.5	8.4	6.8	8.6	7.3	7.7
Lysine[1,2]	4.5	7.7	5.2	6.6	3.1	5.7	5.6
Methionine + Cystine[1,2]	2.2	5.2	6.6	7.4	2.9	4.4	4.2
Phenylalanine + Tyrosine[1,2]	2.5	3.4[a]	4.9[a]	4.0	7.5	8.6	7.9
Threonine[1,2]	2.3	4.7	2.9	3.0	3.3	4.6	4.0
Tryptophan[1,2]	0.6	1.3	1.5	1.3	1.4	1.3	1.5
Valine[1,2]	3.9	4.0	5.7	4.4	5.8	5.2	5.3
Alanine[2]		5.2	4.7	4.1	4.4	5.1	4.7
Arginine[2]		6.7	6.9	6.6	7.9	7.5	8.2
Aspartic acid[2]		7.9	8.6	3.5	11.5	7.3	8.9
Glutamic acid[2]		24.3	20.0	27.8	21.2	18.4	19.5
Glycine[2]		5.6	5.7	4.3	5.4	5.2	5.2
Proline[2]		5.6	6.9	10.0	5.8	6.5	5.7
Serine[2]		4.3	4.4	4.2	4.5	4.9	4.7

[1] Indispensable (essential) amino acid.
[2] All amino acids have been adjusted and are reported as g/100 g protein.
[a] Phenylalanine only, tyrosine not available.
[b] Logie and Milanova (2004) (While this patent information did not specifically used the trademark names indicated here, the descriptions in the patents appeared to describe these materials.)
[c] BioExx (2010).

the 7S protein. Logie and Milanova (2004) reported that up to 15% 12S protein can be present in the precipitated 7S fraction, and up to 5% in the soluble 2S fraction. Logie and Milanova (2004) also found that the amino acid data for the 7S and 12S fractions were very similar. This was not the case for the 7S and 2S fractions (Table 11.3). While the specific protein components have not been determined for all isolates, it is reasonable to presume the soluble isolate is mainly the 2S protein and the precipitated protein is mainly a 7S protein.

The effects of the isolation technique on protein structure have been mentioned in the section on protein isolation. To summarize, high temperatures during oil extraction and high pH values during protein extraction have detrimental effects on protein structure which can influence nutritional and functional quality. Controlling temperatures during oil removal and the use of low pH or salt or water extractions have been used to retain the native protein structure.

11.4 Functional properties

11.4.1 Properties of proteins in isolates and ways to improve them

Utilization of canola proteins depends on more than creating a product that has high protein content and minimal levels of antinutritional factors. There are a number of functional properties that will provide some indication as to how the isolate could be used effectively in foods. Solubility, water absorption, fat binding, emulsification, foaming and gelation are functional properties that can make proteins attractive as food ingredients. In addition, modifications to the proteins have been examined as a way of improving some of these properties.

Solubility and water absorption

The first functional property that is usually examined is solubility, as a number of other properties require that the protein be in solution. The two protein isolates that can be produced from canola differ in terms of solubility. As isolates prepared from the supernatant for protein precipitation contain the 2S albumin, they are expected to be highly soluble. This was true for the soluble protein isolated from a hexane extracted meal (Yoshie-Stark *et al.*, 2008) or a methanol-ammonia-water/hexane meal using alkaline extraction (Xu and Diosady, 1994b). In fact, the value of this isolate is based on its high solubility. This isolate was also effective at fat absorption but poor results were obtained for other functional properties. The Supertein™ protein (primarily the 2S protein) from Burcon NutraScience® is also noted for its high solubility and is recommended for use in beverages (Table 11.4). The Isolexx™ from BioExx® is also noted for being very soluble.

Table 11.4 Properties of commercial canola protein material from available data

	Can Pro IP Insoluble Protein	Can Pro SP Soluble Protein	Vitalexx™[a]	Isolexx™[a]	Puratein®[b]	Supertein™[b]
Source	MCN™, Saskatoon Canada	MCN™, Saskatoon Canada	BioExx®, Toronto Canada	BioExx®, Toronto Canada	Burcon NutraScience®, Winnipeg Canada	Burcon NutraScience®, Winnipeg Canada
Key technical considerations	Low molecular weight	Low molecular weight	Hydrolyzed Highly soluble	Minimal protein denaturation Very soluble	Minimal protein denaturation	Minimal protein denaturation Soluble
Nutritional highlights	Essentially amino acids meet WHO/FAO/UNU (2007) requirements Phytate – 0% Gluconsinolates – 3.44 µ moles/g	Essentially amino acids meet WHO/FAO/UNU (2007) requirements Phytate – 0% Gluconsinolates – 3.82 µ moles/g	Essentially amino acids meet WHO/FAO/UNU (2007) requirements Phytate – 0.27% Gluconsinolates ~0.8 µ mole/g Non GMO	Essentially amino acids meet WHO/FAO/UNU (2007) requirements Phytate <0.05% Gluconsinolates ~0.2 µ mole/g Non GMO	Low in lysine Low phytate GRAS status	Essentially amino acids meet WHO/FAO/UNU (2007) Low phytate GRAS status
Recommended applications	Replace fishmeal, soy protein concentrate and animal protein Animal feed	Use where solubility is important Milk replacers	Nutritional beverages and bars Infant formulas Improve food nutrition	Meat products Beverages and soups Baked goods Dairy products Nutrition bars Dry instant protein drinks	Baked goods Meat substitutes Dressings and sauces	Beverages Confectioneries Aerated desserts

[a] Bioexx (2010).
[b] Burcon NutraScience (2010).

The solubility of the precipitated globulin proteins varies with the isolation methods. Proteins extracted with SHMP and precipitated with acid were reported to have good solubility but poor water absorption (Thompson *et al*., 1982) while proteins extracted in 0.4% NaOH and precipitated using acid were reported to have poor solubility between pH 5 and 7 (Klockeman *et al*., 1997). The precipitated protein isolate from the alkaline extraction of methanol-ammonia-water/hexane meal had very low solubility (2.8%) and poor water absorption (Xu and Diosady, 1994b). It would appear that the conformational changes at high pH values do not favour protein solubility.

The solubilities of proteins prepared using salt extraction and precipitation by dilution were not great either, particularly between pH 3 and 7 (Gruener and Ismond, 1997). While conformational changes with the isolation method are minimal, the fact that the proteins are salt soluble leads to low solubility in a test based on solubility in water. Acylation (both acetylation and succinylation) improved the solubility of this isolate at pH values of 6 and higher, but actually reduced it at lower pH values (Gruener and Ismond, 1997). Hydrolysis of the protein is another way to increase solubility. The Vitalexx™ from BioExx® (Table 11.4) has been partially hydrolysed during isolation and, as a result, is reported to be highly soluble.

Foaming

For proteins to act as foaming agents, they must be readily absorbed at an air-water interface, and then reorganize at this interface to reduce the surface tension and finally form a viscoelastic film at the interface (Moure *et al*., 2006). For this to happen there is a need to have both hydrophobic and hydrophilic areas on the protein surface. In particular the ability to expose hydrophobic groups during the reorganization step is critical to the formation of stable foams (Schwenke, 2001). The protein isolates prepared from SHMP extracted protein were noted for having good whipping capacities (Thompson *et al*., 1982). A dispersion in which half of the egg white was replaced by a 9% dispersion of canola protein produced a meringue equivalent to that from egg white alone.

For some non-canola oilseed proteins, good foaming properties have been reported. A low mucilage flax protein isolate was reported to have better foaming properties than those for a soybean protein isolate (Oomah and Mazza, 1993). The foaming properties of safflower proteins were improved if the protein was recovered at pH 6 compared to pH 5 (Betschart *et al*., 1979). Reducing the phytates and polyphenolic compounds in sunflower protein isolates resulted in improved foaming capacity (Pawar *et al*., 2001). With canola proteins, however, the 7S protein found in most precipitated protein isolates has relatively poor foaming properties (Vioque *et al*., 2000; Malabat *et al*., 2001) and as a result, efforts have been made to improve these properties by modifying the isolated proteins.

Chemical protein modifications have involved acetylation or succinylation reactions with available lysine groups that change the positive charge on lysine to an uncharged or negatively charged amino acid, respectively. With succinylation the increase in net negative charge and subsequent changes in protein conformation improved the surface activity at the air/water interface (Gueguen *et al*., 1990). This resulted in improved foaming capacity and stability. Gruener and Ismond (1997) also reported improved foaming capacity when 61% of the lysine was modified by succinylation or 62% by acetylation; however, they also reported a decrease in foam stability. The change in the net charge and conformation of the protein did not support the production of stable foams. Surprisingly, acylation (both acetylation and succinylation) of flax seeds did not improve foaming capacity (Wanasundara and Shahidi, 1997).

Partial hydrolysis of the protein can also be used to expose hydrophobic and hydrophilic regions; however, improved foaming properties were seen only when the degree of protein hydrolysis (DH) was less than 15%. Vioque *et al*. (2000) found that a low DH (3.1%) produced by treatment with alcalase, improved foaming capacity, but decreased foam stability. In contrast, Chabanon *et al*. (2007) found that foaming properties were improved with hydrolysis, but the improvement was unaffected by increased levels of hydrolysis (between 5 and 15%). At levels of hydrolysis above 15%, foaming properties deteriorated as the smaller size of the peptides produced were less efficient in reducing interfacial tension. Using pepsin and trypsin, it was found that at similar DH values, the 7S protein hydrolysate had better foaming ability than the 2S protein (Malabat *et al*., 2001). This was attributed to the fact that the 2S protein hydrolysates contained more hydrophilic amino acids.

Emulsification and fat binding

The emulsification and fat binding properties have been evaluated for a number of oilseed proteins. The surface properties and flexibility of the protein are critical for using proteins as emulsifying agents (Schwenke, 2001) and emulsification properties are related to the surface hydrophobicity of the protein. Safflower protein isolate had emulsification properties similar to soybean (Betschart *et al*., 1979) while an isolate from low mucilage flax protein was reported to have better fat absorption and emulsification properties than soybean (Oomah and Mazza, 1993). Increased removal of phytates and phenolic compounds during protein isolation has resulted in improved emulsification and fat binding properties (Pawar *et al*., 2001). For sesame protein, higher pH and increased salt concentrations in the emulsion were also shown to improve emulsion activity (Inyang and Iduh, 1996).

The ability of the precipitated 7S canola protein to act as an emulsifier is poor and inferior to that of soybean (Thompson *et al*., 1982; Xu and Diosady, 1994b; Vioque *et al*., 2000; Malabat *et al*., 2001; Salleh *et al*., 2002).

This is, in part, because this protein has low surface activity as the native structure tends to be maintained at surfaces (Krause and Schwenke, 2001). Krause and Schwenke (2001) indicated the 2S albumin protein was highly interactive at interfaces and was more effective as an emulsifier. This conclusion was supported by Yoshie-Stark *et al*. (2008) who found the soluble isolate from ultrafiltration was not only superior to the precipitated protein precipitation but was also better than whole egg. Not all studies have reached the same conclusion. Wu and Muir (2008) produced emulsions with greater surface areas, smaller particle sizes and better emulsion stability using the 7S protein as this protein was more hydrophobic that the smaller basic 2S protein. The soluble protein isolate prepared from methanol-ammonia-water/hexane meal using alkaline extraction exhibited high fat absorption. Inserting a negatively charged region into the 7S improved emulsifying properties (Tandang *et al*., 2005) perhaps due to an increase in solubility that allowed the protein to reach the oil/water interface more effectively.

Treatments used to improve foaming have generally resulted in improvements in emulsification properties as both require surface activity at interfaces. Improvements in emulsion capacity and fat absorption were reported for canola proteins that have been succinylated or acetylated (Gueguen *et al*., 1990; Gruener and Ismond, 1997). While Gueguen *et al*. (1990) also reported improvements in emulsion stability, Gruener and Ismond (1997) found the improvement in stability was only seen at low levels of modification and there was a significant decrease in emulsion stability at higher levels of modification. Hydrolysing canola protein isolates (3.1% DH) was also effective in improving fat absorption, emulsion activity and emulsion stability (Vioque *et al*., 2000). Superior emulsifying activity was found at slightly higher levels of hydrolysis (5% for the 7S protein and 10% for the 2S protein) in the study by Chabanon *et al*. (2007). The degree of hydrolysis was again limited by peptide size as small peptides were less efficient in reducing the interfacial tension as is required at the oil/water interface. Malabat *et al*. (2001) produced 7S and 2S hydrolysates using pepsin and trypsin to DH values of 15%, but neither hydrolysate was able to form a stable emulsion. At this level of hydrolysis, peptides contained an average of 7 to 11 amino acids.

The addition of up to 3% guar gum improved both emulsion activity and emulsion stability (Uruakpa and Arntfield, 2005a) of isolated canola protein. While guar gum does not interact with the canola protein, it was able to increase the surface hydrophobicity of the canola protein in mixed systems at pH 6 and 10 (Uruakpa and Arntfield, 2006a). Emulsifying activity and stability were further improved in the presence of salt (Uruakpa and Arntfield, 2005a). While salt increased the surface hydrophobicity at pH 6, this was not the case at pH 10 (Uruakpa and Arntfield, 2006a), leading to speculation that the role of guar gum in this mixture had effects that went beyond the change in surface hydrophobicity. The increased surface

hydrophobicity that resulted from the inclusion of κ -carrageenan was less than with guar gum (Uruakpa and Arntfield, 2006a), and this was reflected in the emulsion activity and stability data where the improvements were not as great as those seen for guar gum (Uruakpa and Arntfield, 2005a).

Gelation

Globular proteins are able to form gels upon denaturation if protein-protein and protein-solvent interactions are balanced. Denaturation of the protein is usually accomplished with heat. The denaturation temperature for canola protein isolated using salt extraction and precipitation by dilution has been reported to be around 81°C (Léger and Arntfield, 1993), but gelation has been reported at temperatures as low as 66°C (Schwenke *et al*., 1998). As prior processing and the presence of salts and sugars can influence the denaturation temperature, it is not always possible to predict the conditions needed for gel formation without determining the denaturation temperature. The 80 °C heating temperature used in the work of Thompson *et al*. (1982) may not have been sufficient to unfold the protein and this may explain why canola protein gels were not formed in their study. When heated sufficiently to induce denaturation, the canola protein needs to be at a concentration of at least 5.4% to 6% to form a gel, rather than a thick slurry (Gill and Tung, 1978; Léger and Arntfield, 1993). Gels formed with the napin protein tend to be weak, but these poor gelling characteristics can be overcome by binding to the cruciferin if both proteins are present in an isolate (Schwenke *et al*., 1998). The strength of the gels from 7S protein increases with higher protein concentrations (Léger and Arntfield, 1993) and the temperature needed to form a gel decreases (Schwenke *et al*., 1998). Gel strength can also be affected by pH and slightly alkaline conditions (pH values between 6 and 8.5) produce stronger gels (Schwenke *et al*., 1998; Léger and Arntfield, 1993) presumably because the increase in net charge increases interactions between proteins. At even higher pH values, gel strength is reduced but a more elastic gel is created (Léger and Arntfield, 1993). Higher salt concentrations have also increased gel strength but reduced the elasticity of the network (Arntfield, 1996). The presence of phytates and phenolic compounds in the isolate produces weaker canola protein gels (Arntfield, 1996; Rubino *et al*., 1996). A similar observation was noted for gels made from sunflower protein (Pawar *et al*., 2001).

A number of different approaches have been tried to improve gelation properties. In general, moderate levels of succinylation and acetylation improved the gel characteristics, particularly firmness, of heat-induced canola protein gels (Paulson and Tung, 1989; Gruener and Ismond, 1997). The increase in surface hydrophobicity and alteration of the protein charge promoted the formation of a strong network. The effect of these charge modification treatments is linked to pH. For succinylated canola proteins at pH values above 6.5, gels would form only in the presence of salt

(Paulson and Tung, 1989) and following acetylation, the pH where the strongest gels could be formed shifted from a pH of 9 to 6 (Schwenke *et al.*, 1998). Phosphorylation of amino and hydroxyl groups can increase the crosslinking of the cruciferin and thereby produce stronger gels (Schwenke *et al.*, 2000).

Attempts to improve the gelation properties by hydrolysis with ficin, trypsin and bromelin were unsuccessful as gel hardness and elasticity both decreased (Pinterits and Arntfield, 2007) and became weaker with increasing hydrolysis. Disrupting the primary protein structure did not support gel formation. Inclusion of transglutaminase (TGase), a crosslinking enzyme, on the other hand, improved both the elasticity and hardness of the heat induced gels (Pinterits and Arntfield, 2008) and stronger gels resulted at higher TGase concentrations. The combination of limited hydrolysis and TGase produced even stronger gels than the TGase alone (Pinterits and Arntfield, 2007). Gels that mimicked the hardness or elasticity (not both at the same time) of tofu or bologna could be produced (Pinterits and Arntfield, 2008).

Protein-polysaccharide interactions can also be used to improve gel properties. Interactions between biopolymers to form complexes or incompatibility between biopolymers can affect the properties of the mixture. Increased interaction between biopolymers can increase gel strength while exclusion of biopolymers can lead to the individual biopolymers behaving as though they were present at higher concentrations (Tolstoguzov, 1998).

Although guar gum is not compatible with canola protein, a mixture of guar gum (1.5%) and canola protein (20%) at pH 10 in 0.5 M NaCl (the conditions producing the strongest gel for canola protein guar gum mixtures) had lower gel strength and less elasticity than the canola protein alone (Uruakpa and Arntfield, 2005b), suggesting the guar gum was interfering with network formation. Stabilization of the protein structure by the guar gum (Uruakpa and Arntfield, 2006b), may have also inhibited gel formation. In contrast, the addition of 3% κ-carrageenan to 15% canola protein isolate at pH 6 in 0.05 M NaCl improved the strength and elasticity of the gel (Uruakpa and Arntfield, 2004). The ability of κ-carrageenan, a charged sulphated polysaccharide, to form a complex with the canola protein led to the formation of a network that was 3.5 times stronger than for the canola protein alone and 14 times that for κ-carrageenan alone (Uruakpa and Arntfield, 2004).

11.5 Utilization of canola and other oilseed proteins

As proteins are present in many food products, there are many possible applications for using oilseed proteins as food ingredients. Most of the work in using these proteins in food is experimental, and applications will depend on the functional properties already discussed.

11.5.1 Feed

One of the attractive features of the canola protein is its well-balanced amino acid composition as can been seen for commercial protein isolates (Table 11.3). With the exception of the Puratein®, all of the essential amino acid requirements are met. The Isolexx™ isolate from BioExx® is reported to have an amino acid score of ~1.15 (BioExx®, 2010). Not only is this a desirable quality for human consumption, but it is of value in feed rations. While meals rather than isolates are more often used for animals for economic reasons, the aquaculture industry has a need for more processed protein fractions and the highly soluble isolates from MCN have been recommended for use in this application (MCN, 2010).

11.5.2 Foods

A number of studies incorporating canola protein into foods as well as recommended uses from producers of canola isolates are available. In addition, there is work where further processing of the protein results in the production of bioactive peptides with potential health benefits. In addition to addressing these uses for canola protein, the potential for newer protein sources, flax and hemp, will be mentioned.

Food ingredients

The applications of canola and other oilseed proteins as a food ingredient can only demonstrate the potential for these proteins, as commercially available products are still limited. One of the benefits associated with the inclusion of these proteins is the nutritional benefits, where canola has all the essential amino acids, and complements cereals that tend to be low in lysine and legume proteins that are low in the sulphur amino acids.

To improve the nutritional quality of baked goods, studies in which a sesame protein isolate (El-Adawy, 1995) or succinylated sesame proteins (Yue *et al.*, 1991) were included in bread have been conducted. Peanut proteins were used in the preparation of composite flours (Singh and Singh, 1991) and sunflower proteins were included in extruded products (Sotillo *et al.*, 1994). The non-hydrolysed (Isolexx™) and precipitated (Puratein®) proteins, from BioExx® and Burcon NutraScience®, respectively, have both been recommended for use in baked goods (Table 11.4).

The gelling properties of canola isolates led to investigations on using this protein source in meat products. In beef patties, the inclusion of canola protein gave more tender products with higher cooking yield, whereas the same isolate decreased the firmness of wieners with an addition of only 3.4% (Thompson *et al.*, 1982). Succinylated canola proteins were able to better demonstrate the properties needed in comminuted meat products (Paulson and Tung, 1989). Both the Puratein® and Isolexx™ proteins have been recommended for use in meat products but at low concentrations of 1.6–2.4% (Tables 11.5 and 11.6). It has also been suggested that flax proteins can be used in meat emulsions (Oomah and Mazza, 1993).

The use of peanut protein in cheese has also been reported (El-Sayed, 1997), as have flax proteins in ice cream (Oomah and Mazza, 1993). The use of the canola isolate Isolexx™ in dairy products has been recommended at levels of 3–4% (Table 11.5). Other recommended uses for Isolexx™ and Puratein® include nutrition bars, salad dressing and instant drink powders (Table 11.4). For the more soluble Vitalexx™ and Supertein™ proteins, the focus seems to be on nutrition, and applications include beverages, infant formulas, confectionery and aerated desserts (Table 11.4) with levels as high as 90% for dry instant protein drinks (Table 11.5).

Although minor in terms of production, the use of flax and hemp proteins have received some attention. The attraction of these proteins is related to the nutritional value and possible health benefits. In terms of essential amino acids, lysine is the limiting amino acid for both proteins (Table 11.6) and is slightly below the WHO/FAO/UNU (2007) standard.

Table 11.5 Recommended levels of canola protein isolates in various applications

	Vitalexx™[a]	Isolexx™[a]	Puratein®[b]	Supertein™[b]
Source	BioExx®, Toronto, Canada	BioExx®, Toronto, Canada	Burcon NutraScience®, Winnipeg, Canada	Burcon NutraScience®, Winnipeg, Canada
Baked goods – breads, cookies, batters, etc.		0.5–3%	1.2–3.5%[c,d]	1.1–1.2%[e]
Beverages/soups	0.5–2%	0.5–2%	4.5%[c]	2.6%[e]
Dairy products – cheese, frozen desserts, yogurt, etc.		3–4%		
Glaze			21%[c,d]	
Infant formula	3–10%			
Instant drink powders	2–8%	35–90%		
Meats products		2%		1.4–2.6%[e]
Meringue/nougat			3.7%[c]	3.6%[e]
Nutrition bars	5–25%	15–40%		
Salad dressing/ mayonnaise			0.5–1.6%[d]	

[a] BioExx (2010).
[b] While patent information used for these isolates did not specifically used the trademark names indicated here, the descriptions in the patents appeared to describe these materials.
[c] Murray (2005).
[d] Hiron *et al*. (2006).
[e] Hiron (2007).

Table 11.6 Amino acid composition of flax and hemp proteins

Property	WHO/FAO/UNU (2007) indispensable amino acid requirements	Flax protein[a]	Hemp protein[b]
% protein (dry basis)			
Histidine[1,2]	1.5	2.2	3.2
Isoleucine[1,2]	3.0	4.0	3.4
Leucine[1,2]	5.9	5.9	6.5
Lysine[1,2]	4.5	4.0	4.2
Methionine + Cystine[1,2]	2.2	2.8	4.1
Phenylalanine + Tyrosine[1,2]	2.5	7.0	7.3
Threonine[1,2]	2.3	3.7	4.0
Tryptophan[1,2]	0.6	1.8[c]	0.8
Valine[1,2]	3.9	4.8	4.1
Alanine[2]		4.5	5.2
Arginine[2]		9.5	11.4
Aspartic acid[2]		9.7	11.9
Glutamic acid[2]		19.8	19.6
Glycine[2]		4.8	4.3
Proline[2]		3.6	4.8
Serine[2]		4.6	5.3

[1] Indispensable (essential) amino acid.
[2] All amino acids have been adjusted and are reported as g/100g protein.
[a] Oomah and Mazza (1993).
[b] Hemp Oil Canada (2010).
[c] Bhatty and Cherdkiatgumchai (1990).

What distinguishes these proteins is the high levels of arginine (Table 11.6). As arginine is a precursor for nitric oxide production, it may have an effect on immune response and muscle repair and growth. While the flax (Natunola, 2010) and hemp protein (Hemp Oil Canada, 2010) products available are not true isolates (~50% protein), the unique characteristics of these proteins may lead to an increase in their use. Some work has been done on examining and improving the functional properties of flax protein (Wanasundara and Shahidi, 1997).

Bioactive compounds

As noted above, when improving functional properties of canola proteins, there are benefits to using limited hydrolysis. More extensive hydrolysis, however, has produced peptides that have potential health benefits, including antioxidant properties and inhibition of angiotensin I converting enzyme (ACE) which leads to a reduction in blood pressure.

Antioxidant activity, which is often based on a DPPH [2,2-diphenyl-1-picrylhydrazyl] assay, is influenced by the DH and the enzyme used for hydrolysis. With papain and pancreatin, Yoshie-Stark *et al*. (2008) found no

improvement in antioxidant activity following hydrolysis of the 7S and 2S proteins to DH values of 3 and 9%, respectively, whereas Cumby *et al*. (2008), using Flavourzyme, obtained high DPPH values for a hydrolysate with a DH value of 6.3%. This may be due to differences in fragment size rather than the extent of hydrolysis. Higher DH values (20.6% with alcalase and 18.9% with a mixture of alcalase and Flavourzyme) resulted in significantly lower DPPH scavenging capacity (Cumby *et al*., 2008). Canola hydrolysates with DH values of 14 and 30% for alcalase, and a combination of acalase followed by Flavourzyme, respectively, have been shown to scavenge superoxide and hydroxyl radicals, another indicator of antioxidant activity (Xue *et al*., 2009).

ACE inhibition is dependent on the DH. No ACE inhibition was observed with DH values below 9% (Yoshie-Strark *et al*., 2008) and at higher DH values, a correlation between DH and ACE activity has been reported (Wu *et al*., 2009). Several peptides containing only 2 or 3 amino acids were shown to have strong ACE inhibition (Marczak *et al*., 2003). While many enzymes can be used for hydrolysis, the most effective ones in terms of ACE inhibition are Alcalase 2.4 L and Protease M "Amano" with IC_{50} (concentration to reduce ACE activity by 50%) values of 29 and 26 μg protein/mL, respectively (Wu *et al*., 2009). Hydrolysates from a canola protein mixture containing both the 2S protein and the 7S protein (IC_{50} = 15 μg/mL) were more effective than hydrolysates of the purified 7S cruciferin (IC_{50} = 35 μg/mL) and 2S napin (IC_{50} = 29 μg/mL) (Wu and Muir, 2008). Isolation of the spe- cific peptides using immobilized ACE resulted in product with IC_{50} values of ~0.25 μg/mL (Megías *et al*., 2006). As promising as these results appear to be, the drug that is prescribed as an ACE inhibitor, captopril, has an IC_{50} value of 0.0015 μg/mL.

Several other applications for hydrolysed canola protein have been suggested, including inhibition of HIV protease (Yust *et al*., 2004), promotion of cell growth for Chinese Hamster Ovary (Chabanon *et al*., 2008) and production of meat-like flavour compounds (Guo *et al*., 2010). These applications are experimental but help illustrate the potential for this protein source.

11.6 Issues in using canola and other oilseed proteins

One of the attractions in using canola (and other oilseed) proteins in foods is the balanced amino acid composition. The presence of antinutritional factors in the meals that are left over following oil extraction has provided a challenge in using this valuable protein source effectively. Production of protein isolates has not only provided a more concentrated protein fraction, but has removed many of the problems associated with the meal. As a result, canola protein is well positioned for use as a food ingredient. The fact that at least three different companies are actively working with

canola protein isolates would suggest there could be a strong future for this protein.

Utilization of canola proteins in food does require regulatory approval, as these materials have not traditionally been consumed by humans. To gain such status, trials are required that demonstrate the safety of these materials. In the United States of America, the designation 'Generally Regarded As Safe' or 'GRAS' allows a non-traditional product to be used at the level specified, regardless of how it is used. In addition, food additives can be declared safe for specific applications. A self-declared GRAS status is allowed when the scientific data (animal testing data) has been reviewed by an independent panel of scientists and is concluded to be safe. For GRAS status, a petition is submitted to the Expert or GRAS Panel of the Food and Drug Administration (FDA) for review of the scientific data. If there are no objections, the product is given GRAS status. While GRAS status opens up the American market for this product, other countries have their own regulations for using novel ingredients. In the European Union and Canada, these non-traditional protein sources require approval under Novel Food Legislation to ensure they would not be dangerous to or mislead the consumer nor would they nutritionally disadvantage the consumer. As noted below, the companies producing canola protein are located in Canada, but two have initially sought GRAS status in the United States of America. The belief is that the data required for the GRAS petition can also be used when seeking approval in other jurisdictions. This, however, will take time.

The soluble and insoluble proteins from MCN™ Canola appear to be targeted to the feed market. There may be food uses for the soluble protein in beverage applications. The authors of this chapter are unaware of the regulatory status of these products.

Burcan NutraScience® has all the elements in place to produce functional isolates. While the Puratein® is low in lysine, the company has numerous patents and both the Puratein® and Supertein™ have received GRAS status notification in the United States, indicating the antinutritional factors are not a concern. Regulatory approval elsewhere has not been requested at this time. The main challenge in terms of using these isolates is that they are not commercially available for purchase and there is no indication that will change in the immediate future.

By taking the approach of controlling the recovery of both the oil and protein components from canola, BioExx® has produced two isolates for which the protein structure has been retained and the isolates have high solubility, with the Vitalexx™ being more soluble than the Isolexx™ due to hydrolysis of the Vitalexx™ protein during the isolation procedure. They are using a *Brassica juncea* canola which is non-GMO material. BioExx® is in the process of seeking GRAS status and has a facility in Saskatchewan, Canada that should have product available by the fall of 2010 and is about to construct a second facility in North Dakota, USA. Again achieving

GRAS status would be the first step in addressing the regulatory issues related to food safety.

The future for canola protein is bright, but it will take time to realize the potential of these protein materials.

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12

Potato proteins

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Abstract: Increasing population results in an increasing demand for proteins due to their nutritional value. Therefore, new protein sources or protein isolation from existing side-streams is of importance. Recent developments in extraction technologies have resulted in potato protein isolates having high techno-functional and nutritional properties. This chapter will give an update on the different proteins present in potato tuber, their physico-chemical properties and functionalities. Recent developments in their manufacture and large-scale extraction will be highlighted. The technical data of industrially produced potato protein preparations and examples of their use in target applications will be given.

Key words: potato proteins, industrial extraction, functionality.

12.1 Introduction

As the world population increases, so too does the need for welfare in highly populated countries like China, India and Brazil. Consequently, there is an increasing pressure on food production systems to fulfill these increasing needs whilst keeping their environmental impact as low as possible. The demand for proteins is growing especially rapidly due to their nutritional and functional value. Recent developments have resulted in the realization that potatoes can be a valuable source for various protein preparations due to their unique (techno)-functionalities.

Potato, *Solanum tuberosum*, ranked fourth amongst world staple crops in 2008 (FAO, 2008; Hijmans, 2001). The annual production of potato is 322 million tons, while maize, rice and wheat production are 785, 652 and 607 million tons, respectively. Potatoes are, as such, part of the daily menu for many consumers. As well as their direct use as food, however, potatoes are also widely used as a raw material for the extraction of starch. Freshly harvested, they contain about 80% water and 20% dry matter of which

approximately 60 to 80% is starch. This starch is applied as native or modified starch in both food and industrial applications. Annually more than 2 million tons of potato starch is produced and is mainly processed in the EU.

Another ingredient from potatoes that has recently drawn attention is the protein fraction. On a dry weight basis, the protein content of potato is similar to that of cereals and is very high in comparison with other roots and tubers. The potato grows in moderate climates and there are many varieties. Moreover, in combination with the long history of cultivation and their widespread use, potato tubers give an exceptionally high yield per area, from approximately 42 tons (for consumption) up to more than 60 tons (for starch production) per hectare can be produced, which is many times the amount of any grain crop. This makes potatoes a useful raw material for the extraction of protein ingredients. Although, potato protein has been produced as a sideline to starch extraction for years, recent developments in extraction technologies have resulted in protein preparations having valuable techno-functional and nutritional properties. Good quality potato protein can be isolated commercially from the starch industry as a side-product of starch refinery and, to a lesser extent, from the French fries industry where it is derived from the wash water and of the losses from cutting and rasping. Assuming a mean recoverable protein content of 1.2%, the potato starch industry represents a potential of 240 000 tons of high quality protein per year. The protein quality in potatoes varies depending on growth stage, soil and variety. Starch potatoes form a good and reliable source of protein as the starch quality also depends on growing conditions and adequate storage of the potatoes.

This chapter will give an up-to-date review of the different potato proteins present in potatoes and look at their physico-chemical properties and functionalities. Recent developments in their manufacture and large-scale extraction will also be discussed and the technical data from industrially produced potato protein preparations and examples of their use in target applications will be given.

12.2 Physico-chemical properties of the different potato proteins

12.2.1 Potato juice

Within the traditional, potato starch manufacturing process (Swinkels, 1990), potato proteins are present in the potato juice after removal of the starch (also called potato fruit juice, PFJ) and were traditionally considered to be a low value side-stream. PFJ contains 5% (w/w) dry matter (Plieger, 1986), which constitutes approx. 35% proteins, 35% sugars, 20% minerals, 4% organic acids and 6% other compounds such as chlorogenic acid (Knorr, 1977). Chlorogenic acids and other reactive phenolic compounds are critical

for protein recovery. Until recently, potato proteins were recovered using acidic-heat treatment of PFJ. This process results in an irreversible precipitation of the proteins with a total loss of functionality. This explains why, so far, the use of potato protein has been limited to feed.

Soluble proteins present in PFJ juice have been divided into three groups (Racusen and Foote, 1980): patatins (30–40%) (Racusen and Foote, 1980), protease inhibitors (40–50%) (Pouvreau *et al.*, 2001) and other proteins (10–15%) (mainly enzymes like kinases and enzymes involved in starch synthesis) (Pouvreau *et al.*, 2001; Man *et al.*, 1997).

12.2.2 Patatin

Patatin was given its common name by Racusen and Foote (1980). Patatin consists of a family of 40–42 kDa glycoproteins (Pots *et al.*, 1999) all with iso-electric point (pI) values between 4.5 and 5.2. At neutral pH and ambient temperature, patatin exist as a dimer held together by non-covalent hydrophobic forces. Patatin displays a high secondary structure: 35% alpha-helixes, 45% beta-strands and 15% aperiodic (Pots *et al.*, 1998; van Koningsveld *et al.*, 2001). It displays a relatively low denaturation temperature (60°C at pH 7.0; Pots *et al.*, 1998) and a relatively low stability as a function of pH (loss of structure at pH $\leq$ 4.5; Pots *et al.*, 1998). Patatin has a lipid acyl hydrolase (LAH) activity, critical for its functional properties, and was suggested to be part of the defence mechanism (Galliard and Dennis, 1974; Andews *et al.*, 1988).

12.2.3 Protease inhibitors

Protease inhibitors (PIs) are naturally present in many tubers and seeds. They are proposed to be storage proteins (source of sulphur-containing amino acids), which control endogenous proteases or act as a defence mechanisms (Jongsma, 1995). PIs are the most abundant group of proteins present in PFJ (Table 12.1). PIs are a more heterogeneous group of proteins than patatin and are classified into seven different sub-groups based on their intrinsic characteristics (molecular weight (Mw), pI) and their enzyme-inhibiting activity (serine, cysteine, aspartate and metallo protease inhibitors) (Pouvreau *et al.*, 2001).

Recently PIs have been considered not only for their anti-nutritional properties, but also for their potential as anti-carcinogenic agents (Kennedy, 1998), and as positive dietary agents (Hill *et al.*, 1990) via their effect on the release of cholecystokinin (CCK) (Kissileff *et al.*, 1981). They have also been studied for their potential in treating dermatitis (Raffi *et al.*, 1999; Denda *et al.*, 1997; Ruseler-van Embden *et al.*, 2004).

Due to the presence of disulfide bridges (stabilization of conformation), no changes in 2D or 3D structure occur between pH 3.0 and 7.5 (Pouvreau *et al.*, 2005). Potato PIs are considered to be beta-II proteins based on their unusual secondary structure (Manavalan and Johnson, 1987). Interestingly,

Table 12.1 Protein composition of potato juice (adapted from Pouvreau *et al.*, 2001 and 2003)

Protein groups	% in potato juice protein	Mw (kDa)[1]	pI range	Inhibited proteases
Patatin	38	81 (2)	4.5–5.2	None
Potato inhibitor-1	5	40 (5)	5.7–7.8	T, C
Potato serine protease inhibitors	22	20–21 (2)	5.8–6.9	T, C
Potato cysteine protease inhibitors	12	20–23 (1)	5.8–>9.0	T, C, CD
Potato aspartyl protease inhibitors	6	20–22 (1)	6.2–8.7	T, C, Pa
Potato Kunitz protease inhibitors	4	20 (1)	8.0–>9.0	T, C
Other serine protease inhibitors	2	21–22 (1, 2)	7.5–8.8	T, C
Potato carboxypeptidase inhibitor	1	4.3 (1)	?	CA
Other proteins	10	nd	nd	

[1] (number of sub-units).
T = trypsin, C = chymotrypsin, Pa = papain, CD = cathepsin D, CA = carboxypeptidase A.

many protease inhibitors, such as kunitz-type protease inhibitors from soy, belong to the all-beta protein class of which beta-II proteins are a sub-class (Pouvreau *et al.*, 2004).

12.2.4 Other proteins

Proteins from PFJ that do not belong to the patatin family or show protease inhibiting activities are discussed in this paragraph. The total amount of these proteins accounts for about 12% of the proteins present in PFJ (Pouvreau *et al.*, 2001). They have molecular weights higher than 40 kDa and they include lectins (Allen *et al.*, 1996), polyphenol oxidases (Partington and Boldwell, 1996), oxidase, lipooxygenases (Giuseppin and Spelbrink, 2009), enzymes involved in starch synthesis (Marshall *et al.*, 1996) and phosphorylase isozymes (Gerbrandy and Doorgeest, 1972).

12.3 Functionality of different types of potato proteins

The solubility of a protein is considered a key characteristic for its successful application in food, since it is in itself a key parameter and determines to a large extent other techno-functional properties such as foaming, emulsifying and gelling. Protein solubility, in general, is highly influenced by the process history (e.g. heat load, pH, shear stress). Moreover, techno-functional properties strongly depend on the final composition of a protein preparation (e.g. type of proteins, purity, salt content and composition).

Foams

Foam capacity and stability (based on drainage, coalescence and Ostwald ripening) of potato protein solutions have been extensively studied (Boruch *et al.*, 1989; Partsia and Kiosseoglou, 2001; Jackman and Yada, 1988; Ralet and Gueguen, 2001). A fair comparison of the results of foam capacity and stability from various scientific studies is very difficult since the techniques used to make foam vary as does the preparation of potato protein isolates and the fact that each group of potato proteins displays different behaviour. Scientific literature shows that potato proteins perform worse than whey protein isolates (Jackman and Yada, 1988) but better than egg albumins and caseins (Partsia and Kiosseoglou, 2001; Ralet and Gueguen, 2001).

Patatin showed a low foam volume formation at neutral pH (van Koningsveld *et al.*, 2002) but is, on the other hand, very stable against drainage. When the pH became closer to its pI, it resulted in a larger foam volume; however, the foam was relatively unstable (van Koningsveld *et al.*, 2002). The foam capacity of PIs at a neutral pH is higher than that of patatin, but is more unstable. Its foam capacity and stability differ greatly when using sparging or whipping techniques (van Koningsveld *et al.*, 2002). Heat-induced unfolding of potato proteins prior to foam formation did not improve the foam stability of mixtures of patatin and PIs, whereas the foaming properties of a patatin-enriched fraction were improved by this treatment (van Koningsveld *et al.*, 2002). This is important for the practical application of potato proteins, since heat treatment is a standard step in the manufacturing process of most food products, although some food products, like meringue, are heated after the aeration step.

Emulsions

The emulsifying properties of potato proteins have been poorly described in the literature and the lack of conformity within the experiments makes it difficult to compare the results of different studies (Ralet and Gueguen, 2000; van Koningsveld *et al.*, 2006; Holm and Eriksen, 1980). Potato proteins were described as a better emulsifier than WPI (Ralet and Gueguen, 2000), casein (Ralet and Gueguen, 2000) and soy protein (Holm and Eriksen, 1980), especially when using the group of PIs.

Emulsions, prepared on the basis of a patatin-rich fraction, displayed droplet aggregation and appeared to cream relatively fast. These results are thought to be linked to the LAH activity of patatin. The average droplet size of emulsions made from potato proteins (a mixture of patatin and PIs) is determined by the lipolytic release of surface-active fatty acids and monoglyceride from the oil fraction (Van Koningsveld *et al.*, 2006). Emulsions, based on the PIs fraction, are more stable than those of patatin, especially at low pH values (Van Koningsveld *et al.*, 2006).

Gelation

The literature on the gelling behaviour of potato proteins is very scarce. The aggregation behaviour of patatin has been extensively studied (Pots

et al., 1999) but almost nothing is known about the gelation or aggregation behaviour of the PIs fraction. Some preliminary results have shown the importance of the disulfide bridge(s) in PIs during heating. Reshuffling of the disulfide bridges occurs during heating and soluble aggregates (of four molecules) were reported to have been formed (Pouvreau *et al.*, 2005).

The lack of results on the gelling behaviour of potato proteins can be explained by the fact that gelation experiments require a much higher amount of the individual potato proteins: patatin and PIs. Until recently, due to the lack of suitable technologies, to our knowledge, no large-scale purification of potato proteins has been attempted, which limits the research in this field.

12.4 Potato protein isolation

The isolation of potato protein has been studied for several decades. Many routes have been explored with the aim of recovering the proteins with the maximum nutritional and functional properties in the most efficient way.

Most of the routes described are based on laboratory-scale procedures ranging from various adsorption techniques using hydrophobic interaction columns, to combinations of ion exchange techniques (van Koningsveld *et al.*, 2001). Knorr (1977) described various methods to recover proteins from PFJ streams. This is still the basis of industrial practice in the potato starch factory.

To obtain highly functional potato protein, the process has to be designed to meet various requirements in terms of isolation, fractionation and costs. The best functional potato protein is isolated from fresh PFJ. Predominantly PFJ is obtained industrially by the rasping of a mature starch potato and the removal of the starch and the majority of fibres. As previously discussed, the resulting PFJ contains 4–5% dry matter. About 1–1.4% of the PFJ is protein with a molecular weight of >10 kDa and between 0.4 and 0.6% consists of free amino acids.

12.4.1 Instability

PFJ is highly unstable after rasping. The concomitant presence of oxidative enzymes, such as polyphenol oxidases, oxidases and lipoxygenases, and the abundant amount of polyphenolic substrates, such as chlorogenic acid, cause the PFJ to rapidly darken and modifies the proteins. This can result in the flocculation of potato proteins (Prigent, 2005). Many of the colour-forming reactions create compounds which include undesirable flavours.

There are different approaches (or combinations thereof) which improve the stability of PFJ. The strongest effect is obtained by the addition of sulphite as an inhibitor of polyphenol oxidase in combination with oxygen scavengers. Lowering the pH also slows down various enzymatic reactions.

Patatin especially, however, tends to unfold at a low pH and the resulting protein precipitation limits its industrial use. The level of ascorbic acid added or endogenous to the PFJ can also improve the stability. The instability of the PFJ proteins necessitates a rapid processing and rescuing by fractionation into two or more protein fractions.

12.4.2 Fractionation

Potato proteins are classified into two groups that have a tendency to aggregate and flocculate when they are processed (together). The resulting flocculate has poor solubility and functionality in terms of gelling, foaming and emulsification. Fractionation of PFJ has been pursued in various processing designs, such as heat fractionation and ion-exchange. A carefully chosen fractionation method can also be used to obtain fractions with specific physicochemical functionalities. Fractionation methods aim to separate proteins on the basis of their molecular weight, e.g. ultrafiltration (Edens *et al.*, 1997; Poulsen and Koops, 2002), extreme charge (van Koningsveld *et al.*, 2001), hydrophobicity (Racusen, 1989) or their heat stability (Knorr, 1977). A more recent approach has been the use of mixed-mode ligands to combine hydrophobicity with the separation of proteins at a specific iso-electric point (Giuseppin *et al.*, 2008; Lihme *et al.*, 2008). Recent developments enable the application of this combination at an industrial scale, providing the opportunity to isolate potato proteins into high and low molecular weight fractions.

12.4.3 Glycoalkaloids

Glycoalkaloids are common constituents of Solanaceae plants which include courgettes, paprika, aubergines and tomatoes. The glycolysated forms are bitter and slightly toxic compounds which have to be removed to levels comparable to those in consumption of potatoes on a dry weight basis.

In recovering proteins, process conditions are carefully chosen to prevent precipitation of glycoalkaloids onto the proteins. In the case of heat coagulation and concentration by ultrafiltration, glycoalkaloids are accumulated and have to be extracted using acid and/or organic solvents (e.g. Kemme-Kroonsberg *et al.*, 1997).

During ion-exchange and mixed mode processing, the excess of glycoalkaloids can be removed by ultrafiltration. Final glycoalkaloid removal from soluble proteins can be performed with specific or aspecific adsorbents (Giuseppin and Laus, 2008).

12.4.4 Adsorption processes

Adsorption processes offer the advantage of isolating the protein fraction from a mixture, thereby preventing its modification by all kinds of enzymatic and chemical reactions. Low flow rates in classical chromatography

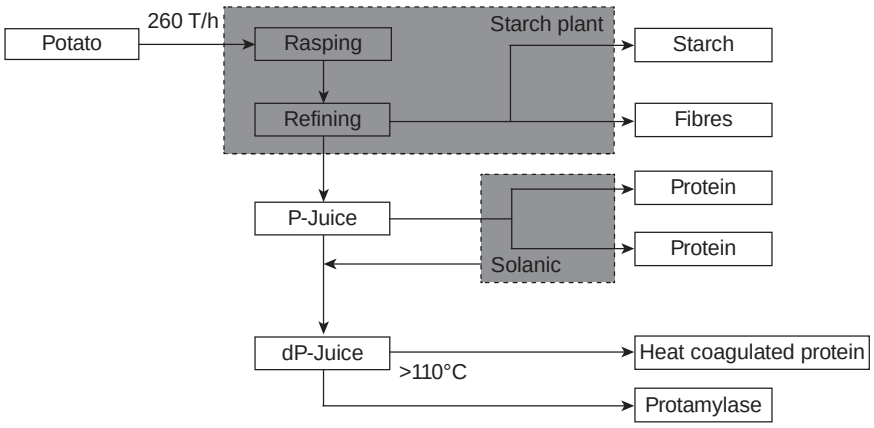


Fig. 12.1 Process scheme of potato protein recovery at AVEBE/Solanic.

led to very slow adsorption processes designed to avoid undesired reactions, but more recent technologies have become available for large industrial-scale adsorption, which enable rapid and cost-effective protein isolation from PFJ.

Scaling-up of the adsorption processes used for food-grade and large-scale processing has been limited until recently by fouling, low productivity and the cost of the resin. Expanded bed adsorption with heavy adsorbents has been designed for an efficient recovery and fractionation of potato proteins (Lihme *et al.*, 2008). Expanded bed adsorption allows an upflow operation and reduces fouling since particulates in the PFJ can now flow through.

A combination of expanded bed adsorption and simulating moving bed technology is used to produce industrially fractionated, highly purified potato protein isolates (Giuseppin *et al.*, 2008; Bisschops and Giuseppin, 2008). Currently, a pilot factory, with a nominal capacity of 1000 tons purified protein per year, is operational in The Netherlands. The integrated process scheme of that factory is shown in Fig. 12.1.

12.5 Specifications of industrially produced potato protein preparations

12.5.1 Potato protein preparations

An overview of the currently available potato protein preparations is given in Table 12.2. Of the listed products, only Heat Coagulated Potato Protein (HCPP) and Fractionated Potato Protein Isolate (FPPI) are commercially available in substantial quantities. HCPP is available in feed grade quality and applied as such. FPPI is made for food and pharmaceutical applications.

Table 12.2 Overview of industrial potato protein preparations

	HCPP	HFPP	UFPP	Strong IEX	PPI	FPPI
Main technology	Heated 90–105 °C	Heated ~60 °C	UF	Strong ion exchange	IEX or mixed mode	Mixed mode
Protein content	60–75%	70–80%	70–80%	>95%	>90%	>90–95%
Energy costs	High	High	Medium	Medium	Low	Low
Chemical costs	Medium	Medium	Low	High	High to medium	Low
Solubility fraction	<5%	Low	Low	Medium	Medium	Medium
	–	High	–	High	High	High
Biological functionality	Low	Only heat stable activity	Medium	High	High	High
Physical functionality	Low	Partly	Partly	Partly – high	High	High
Scalability	Good	Good	Medium	Poor	Poor	Good

HCPP Heat coagulated potato protein.
HFPP Heat fractionated potato protein.
UFPP Ultrafiltrated potato protein.
PPI Total potato protein isolate.
FPPI Fractionated potato protein isolate.

Table 12.3 Characteristics of AVEBE/S *Solanic FPPI* proteins

	Solanic HMW	Solanic LMW
Molecular weight of proteins (kDa)	>35	>4 and <35
Solubility 100% (g/l)	>50 @ pH>6.5	>250 @ pH<4.5
Protein content (% on dry matter)	>92	>95
Ash level (%)	<4	<3
Iso-electric points (pI)	<6.0 mainly 4.8–5.2	>6; majority >8
Charge at common applications	− charged	+ charged

The other products are in a prototype or design stage and may be hindered by scale-up and environmental issues.

The main technical specification is protein content and amino acid composition for nutritional purposes. Physical and chemical functionalities for native proteins are specified in more detail. Heat-treated potato proteins have only limited physical functionality.

The native potato protein isolates are highly varied in their functional properties. The applied fractionation ensures that proteins with opposite charges are separated and therefore do not cause flocculation resulting in a loss of functionality. The main characteristics are given in Table 12.3.

12.5.2 Nutritional value

The nutritional value of potato protein isolates and fractions differ considerably from those of the whole potato. This difference in amino acid composition is common to roots and tuber crops with a high water content as opposed to proteins originating from seeds. In a potato, aside from 1–1.4% of protein with a molecular weight larger than 10 kDa, about 0.4–0.6% of free amino acids and small peptides are present with a different amino acid composition. This fraction is relatively rich in glutamate, aspartate and asparagine (e.g. Hughes, 1958). Nutritional comparisons can be made based on different classes of amino acids. The nutritional value expressed as essential amino acids is given in Table 12.4. This table shows that potato protein isolates have a balanced composition specified in many amino acid clusters. Each class is relevant to the target age groups and diets.

In formulating optimal amino acid diets, various nutritional parameters have been developed. The main parameters are: Protein Efficiency Ratio (PER), Biological Value (BV), True Digestibility (TD), Amino Acid Score (AAS) and Protein Digestibility Corrected Amino Acid Score (PDCAAS = AAS × TD). The actual target values of these parameters depend on the specific target group.

Potato proteins have a TD of more than 0.98. Table 12.5 shows that the various nutritional parameters of potato protein isolates are high in comparison to other proteins.

Table 12.4 Comparison of key amino acid clusters

g/100 g protein	Solanic HMW	Solanic LMW	Solanic total isolate	WPI	Ca-Cas	Egg white	SPI
Lysine	7.2	7.6	7.3	10.2	7.6	6.8	6
Branched amino acids	20	23	22	23	19	20	17
Essential amino acids	50	52	52	51	46	50	45
S-containing met + cys	3.4	3.9	3.7	5.1	3.2	6	2.5
Aromatic phe + tyr	11.7	12.1	11.6	6.9	10.4	9.7	8.9

WPI Whey protein isolate.
Ca-Cas Calcium caseinate.
Egg white Egg albumin.
SPI Soy protein isolate.

Table 12.5 Nutritional aspects of potato protein isolates FPPI compared with other commercial proteins*

	PDCAAS[#]	PER	AAS	BV
Whole egg	1.19	3.8		1.0
Cow's milk	1.21	3.1		0.84–0.88
Casein	1.23	2.9	1.24	0.88
Soy	0.91	2.1	0.96	0.77–0.84
Wheat	0.43	1.5		0.59
Potato Solanic**	0.99	2.3	1.09	0.99

[#] Reference amino acid pattern is that of the FAO/WHO for the pre-school child.
* According to Schaafsma (2007).
** HMW AVEBE/Solanic product analysis.

Potato protein isolate shows the highest PDCAAS and PER of the vegetable proteins and has a relatively high biological value (BV). Although egg protein has the highest BV of 1.0, the potato protein isolate is not far behind and has a BV value in the same range as high-end animal proteins.

12.6 Uses and applications

Technological progress has recently been made in recovering potato proteins on an industrial scale using a much milder isolation process

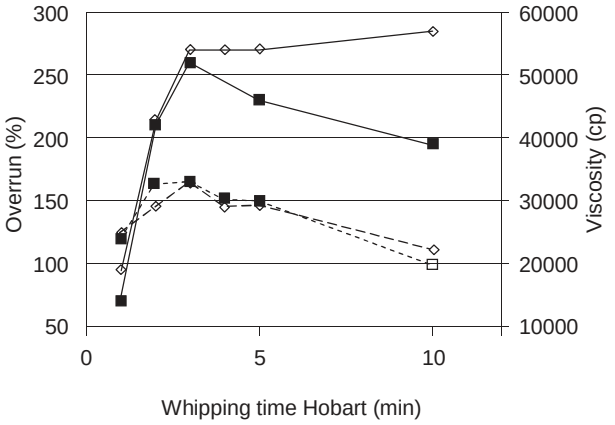


Fig. 12.2 Effect of whipping time on overrun at pH 7 (lower plots LMW and egg albumen) and viscosity (upper plots LMW and egg albumen) of a foam of commercial egg albumen compared to commercial potato protein (LMW fraction). Recipe included 3.1% protein and 75% sugar.

(Giuseppin *et al.*, 2008). These proteins are relatively high in solubility and much more functional with respect to gelation, foaming and emulsifying properties than previous generations of isolated potato proteins.

12.6.1 Foaming properties

There are a variety of foamed foods, such as meringue, angel kisses, sorbet and cakes. Milk proteins or egg white are often used as aeration agents. Potato proteins, however, have also shown foaming properties as previously discussed (Ralet and Gueguen, 2001; Koningsveld *et al.*, 2002) and could be used as an alternative to animal proteins. Tests with Solanic LMW fraction showed that this protein preparation gives similar foaming properties compared to egg albumin (Fig. 12.2). The viscosity of the LMW-foam was also a little higher after an increased whipping time compared to egg albumin. The LMW fraction can also be used for foaming at a low pH with or without heat treatment. This could, for example, be interesting in aerated fruit chews.

12.6.2 Emulsifying properties

The emulsifying properties of a vegetable protein can be of interest for food applications such as dressings, ice cream, emulsified meats and coffee creamers. As previously discussed, the two fractions of potato proteins are: high molecular weights (HMW), which contain mainly patatin, and low

molecular weights (LMW) which contain predominantly PIs. The dominant proteins PIs and patatins have shown emulsifying properties in model systems (Koningsveld *et al.*, 2006; Lokra *et al.*, 2008, 2009; Ralet and Gueguen, 2000). Only a limited number of papers have been published on the topic and variable results were reported.

Commercially available potato proteins (Solanic LMW) have been used to make emulsified food systems, e.g. a dressing. In this case, it was possible to use the potato protein as a single emulsifier in an emulsion with 30 and 50% oil at a pH below 4. The dressings could be processed at both hot and cold temperatures when this potato protein preparation was applied. The optimum results were achieved when this potato protein preparation was heated before emulsification (Giuseppin *et al.*, 2010). Thus, the preceding heat treatment of the protein solution enhanced the functionality of the protease inhibitor fraction and confirmed the results of the fundamental study by van Koningsveld *et al.* (2001). Besides the emulsification properties, the results also showed that the heat treatment especially enabled a reduction of the thickener content in the dressing. The solubility of the LMW fraction was not much affected by the heat treatment. The result in each case was a dressing with a very smooth texture (no granularity). The dressings in which the protease inhibitor was applied as emulsifier were just as stable as the dressings in which egg yolk was used. These results mean that it is possible to make a dressing entirely from vegetable ingredients with potato protein as the emulsifier. The excellent emulsifying properties of the protease inhibitor fraction at a low pH can also be used for emulsion-based beverages or other emulsified low pH applications.

Both the commercial protease inhibitor fraction and the commercial patatin fraction were used to prepare a fully vegetable lactose-free ice cream with fat levels varying between 8 and 16%. Preparation of sorbet-type ice cream (at a low pH) with potato protein as the foaming agent is also a possibility. The ice cream with potato protein has a neutral taste and was comparable with that of ice-cream made with milk proteins except that it was missing the typical milk taste, as expected. Potato proteins can also be used for their emulsification properties in creating a vegetable topping for various desserts.

12.6.3 Coacervates with low molecular weight (LMW) fraction

The LMW fraction of potato proteins has a high iso-electric point (see Table 12.3). Most food proteins have an iso-electric pH of approximately 4–5. This unique feature of the potato LMW fraction could be used to make coacervates with anionic polysaccharides like pectin or xanthan gum. It was indeed possible to make stable coacervates with potato PIs at pH 4 and 7 in combination with low-methylated pectin. These coacervates were still stable after heat treatment at 80 °C. These coacervates can be used to encapsulate flavour or other sensitive ingredients.

12.6.4 Gelation properties

Both the patatin and the Solanic LMW fractions can form a gel under specific conditions (Creusot *et al.*, 2010; Giuseppin and Bakker, 2008). The gel formation properties of potato proteins can be used in applications such as brine injected meats, surimi or vegetable meat substitutes. The denaturation temperature of patatin is approximately 20 °C lower compared to those of other food proteins such as ovalbumin, soy glycinin and beta-lactoglobulin. However, the gelling behaviour of patatin, with respect to ionic strength and protein concentration, was quite similar to those of ovalbumin and beta-lactoglobulin (Fig. 12.3). The LMW fraction gelled most effectively at a low pH (<pH 4.5) and with some salt added (more than 0.35% NaCl). A gel could still be obtained at higher concentrations (12% protein) at a pH of 5.4 (highest pH tested) (Giuseppin and Bakker, 2008).

12.6.5 Combined emulsification and gelation properties

The combination of gelation and emulsification can be used in, for example, emulsified meat products. Figure 12.3 shows the texture of a simple emulsified and gelled emulsion which contains almost 50% sunflower oil. The HMW fraction formed a relatively strong gel (expressed as max force in Fig. 12.4), in the emulsified and heated example. The LMW fraction gelled upon heating the emulsion, but the gel strength was lower. Protein preparations like soy protein isolate and sodium caseinate either did not form a gel at all or formed only a very weak one.

12.7 Regulatory status and safety

The various potato protein products have a regulatory status based on the type of processing used. Potato proteins have a long history of safe use in many areas in the world.

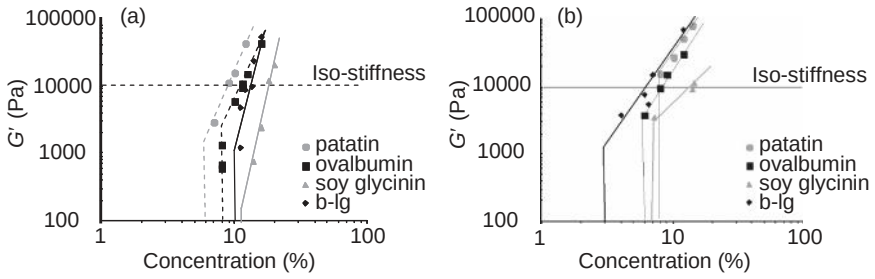


Fig. 12.3 Storage modulus *G´* after completion of the temperature programme as a function of protein concentration of gels formed at pH 7.0, in water (a) and in 100 mM NaCl (b). The lines are guides to the eye. Vertical lines indicate the minimum gel concentration of the proteins. From Creusot *et al.* (2010).

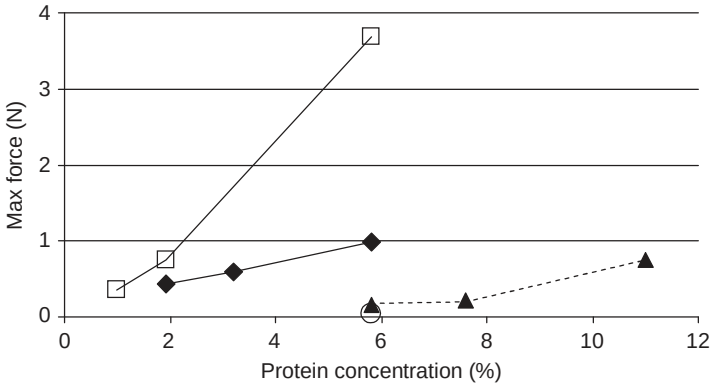


Fig. 12.4 Maximum force in compression test as a function of protein concentration at pH 6.0 with 1% salt. For the HMW fraction ( ) a HMW:oil:water ratio of 1:50:50; 1:25:25 and 1:8:8 was used, for the LMW fraction (♦) 1:25:25; 1:15:15 and 1:8:8 and for soy ( ) 1:8:8; 1:6:6 and 1:4:4. For Na-caseinate 1:8:8 (o) (but this did not give a gel).

12.7.1 Regulatory status

Potato protein products and hydrolysates derived thereof, more specifically, heat coagulated proteins using the AVEBE process, have obtained the GRAS status in the USA (GRAS Approval, 2002) and have been approved as Novel Food in the EU (European Decision, 2002; Novel Food, 1997). The extensive dossier proved the safe process without significant amino acid modification such as the formation of lysino alanine and reduced nutritional value. For both GRAS and Novel Food the limits have been set for high dosage food applications for residual sulphite (<100 ppm) and glycoalkaloids (<150 mg/kg product).

Potato protein isolates using mild separation techniques do not lead to a significant change in the nutritional value, its metabolism or contaminants. Potato proteins are not Novel Food ingredients, but consumer products containing elevated levels of potato proteins, especially specific subfractions, may be considered Novel Food.

12.7.2 Safety

Daily intake

Potato protein intake is related to potato consumption. In Europe the mean daily intake is 275 g potato products with a mean intake of 1100 g potato products in Poland. This corresponds to a potato protein intake of 3.3–13 g potato protein per day and is therefore a significant contribution to the protein requirement for an adult of 34–60 g protein per day which follows the WHO and US RDA guideline of 0.45–0.8 g of protein per kg body weight.

Digestibility

The true digestibility of potato protein is affected by the processing. Heat coagulated protein has a digestibility of 84% (Camire *et al.*, 2009) and can reach up to 98% for an optimized heat coagulation used by AVEBE (Boison *et al.*, 2000).

Allergenicity

The consumption of potato shows the lowest incidence of allergenicity of both vegetable and animal protein sources. This is a consequence of the fast and high digestibility of potato proteins. Skin prick tests on sensitive infants showed an incidence of less than 5% allergenicity for potato compared to 15% and 9% for eggs and cow's milk respectively (Majamaa *et al.*, 2001). In the general population an incidence of less than 1.2% is estimated based on a study of sensitive patients in Korea (Lee *et al.*, 2006).

Glycoalkaloids

Glycoalkaloids are bitter components which may cause adverse reactions such as nausea at low concentration and vomiting at a high conentration. A few poorly documented reports showed toxification with potatoes that may relate to high glycoalkaloid levels. The glycoalkaloids of the potato consist of the triglycoalkaloids (TGA) α-solanine and α-chaconin with an allowed level of 200 mg/kg potato fresh weight. This corresponds to 800 mg TGA per dry matter. Some countries such as the USA are considering reducing the maximum level to 400 mg TGA per dry matter. The mean daily intake in the EU ranges from 55 to 220 mg TGA without significant effects.

In the potato protein produced by AVEBE, which has been approved by the US and EU regulatory authorities, a maximal level of 150 mg TGA per kg HCPP is set.

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13

Mycoprotein: origins, production and properties

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Abstract: This chapter reviews the origins of mycoprotein and its use in creating meat-free products sold under the Quorn™ brand. Production of mycoprotein by fermentation is outlined together with the process for the conversion of harvested mycoprotein into meat-like textures. The underpinning science behind the creation of meat-like texture from mycoprotein is then reviewed together with nutritional properties and clinical research into the way that a diet rich in mycoprotein can impact on public health issues of heart health and obesity. Finally, emerging evidence is presented that shows how the conversion of starch into mycoprotein is environmentally more benign when compared with animal protein, and thus how the original 1964 vision of creating a new protein to address food security may be coming full circle.

Key words: mycoprotein, Quorn, fermentation, texture, sustainability.

13.1 Introduction

13.1.1 Origins and discovery of mycoprotein

The United Nations 1960 projection for the world population predicted a population growth leading to world famine by the 1980s. Books such as *Famine 1975!* (Paddock and Paddock, 1967) suggested disaster would be on the world even earlier. The late Lord Rank, who as J Arthur Rank had created the Rank Film empire, was then chair of the Rank Hovis McDougall (RHM) group of companies and felt that something needed to be done. RHM as a major producer of cereals produced starch as a by-product. The ideal process therefore would be one that converted abundant carbohydrate into the scarce commodity protein (Angold *et al.*, 1989).

Microorganisms have been used by man for centuries but principally to modify or prepare foods. The development of a microorganism as a direct, primary food for man was a brave step, but so the search began.

According to Angold *et al*. (1989) the search for a suitable microorganism began in a sports field alongside RHM's wheat starch plant in Ashford, Kent. This field was occasionally sprayed with surplus starch and the theory was that this would help to select for those organisms capable of using starch as a substrate. This proved to be correct and a strain of *Pennicillium* (coded C1) was isolated with an amino acid profile close to casein. Work progressed on C1 for 3 years but ultimately it was its inability to grow satisfactorily in continuous culture that resulted in the search for a new organism.

The new search began in 1967 with over 3000 soil samples taken from around the world. Ironically, the organism selected *(Fusarium graminearum code A3/5)* came from a garden in Marlow, Buckinghamshire, within four miles of the Lord Rank research centre where the project was located. This was later reclassified as *Fusarium venenatum* (PTA 2684) (Yoder and Christianson, 1998) and is a member of the ascomycota branch of the fungi family, alongside morels or truffles. Mycoprotein can be regarded as the food derived from the mycelium of this *fusarium*.

Work now focused on the technical development of the fermentation process and the testing of mycoprotein for food safety. Initial scale-up studies were carried out using shake flask culture, building to an initial 1300-litre fermentation pilot plant. However, RHM soon realised that it lacked the core fermentation skills to develop the scale required for commercial production and in 1973 formed a three-year collaboration with Du Pont whose chemical engineering expertise led to significant advances. However, it was the formation of a collaborative partnership with ICI in 1984 (Marlow Foods) that made possible the development of the current 180 m^3 scale air lift fermentation technology.

Alongside development of the fermentation technology, studies were also being carried out on food safety (Wiebe 2001, Wiebe 2004) for what was deemed 'the first new food since the potato'. These studies ran to some 26 volumes and two million words, taking two years for the then Ministry of Agriculture Fisheries and Food to read and with initial clearance for sale as a human food granted in 1980 (see Section 13.5).

13.1.2 The development of Quorn™

The technology in isolating an organism capable of turning starch into protein and developing the technology for scale-up was a major challenge. Equally challenging was the development of the technology that enabled the creation of pleasant meat-like textures from mycoprotein and its marketing as a new food. After much consideration, the brand Quorn™ was created as a vehicle to develop and market foods containing mycoprotein. It is a matter of record that the first retail Quorn™ product was the Sainsbury's Quorn™ savoury pie thanks in no small measure to the interest and support of Sir John Sainsbury himself.

Since then Quorn™ has grown to become the world's leading meat-free brand with retail sales of over £200m in ten countries worldwide – quite a journey for a member of the fungi family commonly occurring in soil but first found in 1967 in a field in Marlow, Buckinghamshire.

13.2 Manufacture of mycoprotein

13.2.1 Fermentation and the production of mycoprotein

F. venenatum for mycoprotein production is grown under strictly defined conditions, with temperature, pH, nutrient concentration, dissolved oxygen and growth rate all maintained constant (Trinci, 1991). RHM had used stirred tank fermentation for scale-up. However, because of their filamentous morphology, cultures of fungi are more viscous than bacterial cultures (Righaleto, 1979) and are more difficult to mix. This was eventually solved by the formation of a joint venture with ICI in 1984 and the development of the current air lift fermentation technology.

Figure 13.1 depicts the manufacturing process for the production of mycoprotein. Freeze dried stock cultures are stored in repositories worldwide (*Fusarium venenatum* ATCC PTA-2684). The freeze-dried organism is first revived and grown in a mini lab fermenter during which time the main air lift fermenter is sterilised and made ready for inoculation.

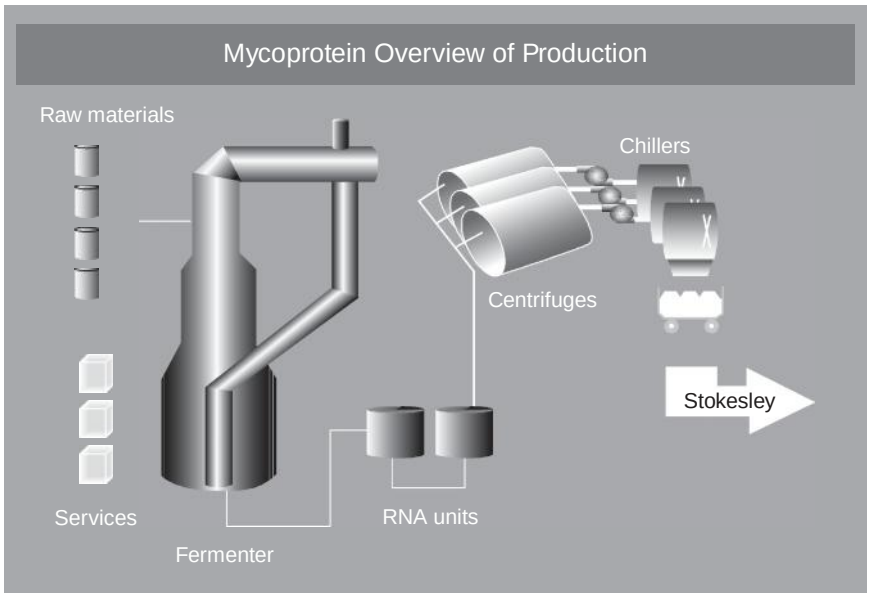


Fig. 13.1 Production process for mycoprotein.

Inoculation is into glucose as the fermentation medium with control of pH, the addition of trace minerals and vitamins for healthy growth and the presence of ammonia as a nitrogen source. After inoculation the organism enters its growth phase with air pumped into the base of the fermenter causing the characteristic 'lift' and recirculation to the point where the loss of entrained air causes the liquid to fall until it picks up more air and the 'air lift cycle' is carried out once again. This is carried out until a suitable level of recirculating solids is reached. At this point harvesting of the mycoprotein begins and then continues in steady state continuous flow for up to six weeks. At first the fermentation liquid is heated to cause a reduction of intrinsic ribonucleic acid (RNA) (Ward, 1996). The rapid heating of the fermenter broth stops growth, disrupts ribosomes and activates endogenous RNAases which break down cellular RNA to 5′ nucleotides which then diffuse through the cell wall reducing intrinsic RNA content in the mycoprotein to *ca*. 1% (w/w), a level similar to animal liver and well within the upper limit of 2% recommended by the WHO (Trinci, 1992). These are removed, concentrated and have found commercial use as a flavour enhancer – Mycoscent™, particularly effective in salt reduction of certain foods (Rodger *et al*., 1998). After RNA reduction the fermenter broth is clarified by centrifugation. The resultant solids are further concentrated by vacuum chilling resulting in mycoprotein at *ca*. 24% (w/w) total solids. This material has the appearance of bread dough but lacks the elasticity associated with gluten mass. Mycoprotein at this point is ready for conversion to the meat-like texture characteristic of Quorn™ foods.

According to Trinci (1992), in a continuous flow culture, growth of the fungus can be restricted by the supply of any nutrient but is usually limited by the concentration of the carbon and energy source (e.g. glucose) with all other nutrients present in excess. However, continuous flow cultures avoid the fluctuating conditions inherent in batch cultures (Pirt, 1975) and enable perpetual exponential growth of the organism to be maintained at a specific growth rate approaching its maximum rate of growth for the prevailing conditions. In practice, mycoprotein fermentations are run for about six weeks, which yields a productivity some five-fold greater than could be achieved by a series of separate batch fermentations (Sadler, 1988).

Figure 13.2 shows the microscopist's view of mycoprotein and demonstrates the filamentous and branched nature of the hyphae. Indeed, as we will see later on, it is this filamentous nature which is so important in the ability of mycoprotein to create meat-like texture.

13.3 The production of foods from mycoprotein

Figure 13.3 depicts the production process for the creation of Quorn™ mince and pieces from mycoprotein.

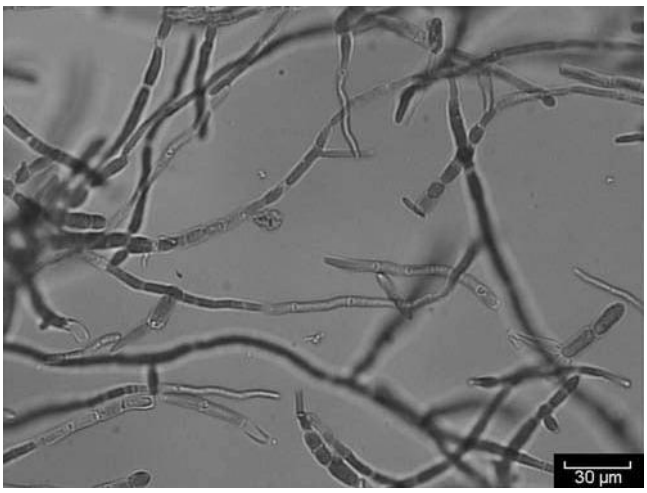


Fig. 13.2 The filamentous nature of mycoprotein.

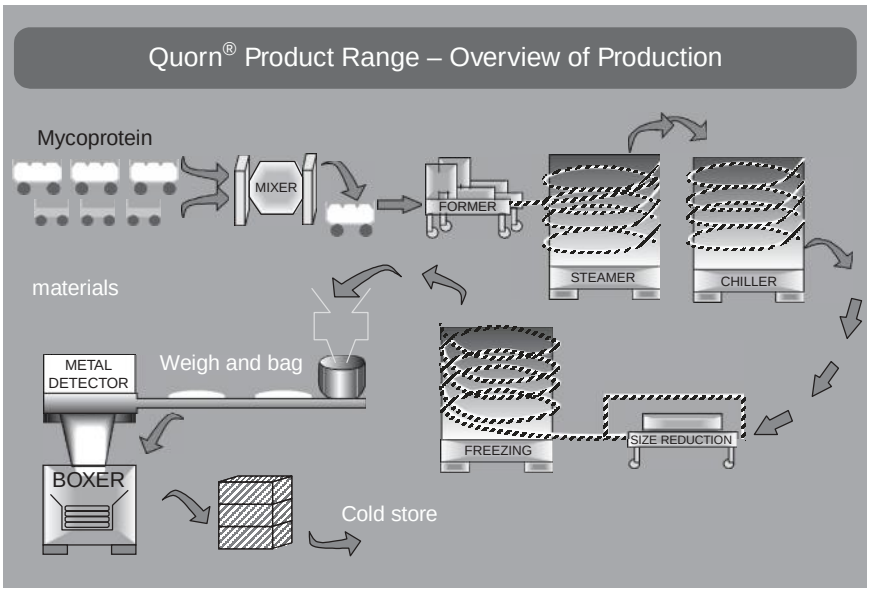


Fig. 13.3 The production process for Quorn mince and pieces.

From receipt of mycoprotein the production process is batch continuous. In the production of Quorn™ mince, mycoprotein is mixed with a little egg albumen, some roasted barley malt extract and water. The process for Quorn™ pieces is similar at this point except no malt is added but a natural flavouring is mixed in to give a savoury character. Mixing is carried out at

low shear and mix sizes approach two-tonne batches. The apparent simplicity of this process masks the challenge of dispersing a powder of complex hydration characteristics such as egg albumen into a viscoelastic material such as mycoprotein.

After mixing, the mass is discharged into equipment common to the food industry used to produce shaped blocks by forming under pressure. It is at this point that the flow characteristics of the mix begin to introduce laminations which can be considered as textural pre-cursors for the final meat-like textural attributes achieved in the final product. The formed blocks are then raised to 90 °C by live steam. This denatures the egg proteins which begins to 'fix' the textural precursor introduced on forming.

The blocks are then chilled and frozen to approx −10 °C. Freezing is carried out over *ca*. 30 minutes in order to achieve a controlled and slow rate of freezing relative to many other foods. Further changes in sensible heat during subsequent frozen storage result in temperatures of *ca*. −21 °C. Freezing is a critical process in the creation of the desirable meaty texture. The controlled growth of ice crystals effectively pushes the filamentous hyphae together to create fibrous bundles with a resultant texture that can be described as meaty. In addition, this finished product texture is remarkably stable both to end-use environment, e.g. pH and processing (Knight *et al.*, 2001), allowing relatively unconstrained food product development.

Thus, at face value we have a straightforward process definition of mix, form, cook and freeze. However, the underlying science is far more interesting than that.

13.4 Texture creation in mycoprotein

Figure 13.2 has already shown us the filamentous structure of mycoprotein. This filamentous nature is not typical for ascomycota where many within the family tend to a globular morphology, and indeed after *ca*. 30 days of steady state fermentation and harvesting some globular forms of *fusarium venenatum* appear (Simpson, 1996), at which point the fermentation is terminated, as this morphology produces an inferior finished product texture. In fact, as we shall see, the filamentous nature of mycoprotein is prized for its ability to create pleasant meat-like textures.

For Quorn™ pieces and mince, microstructural analysis shows an entangled mass of mycoprotein hyphae with at least some gelled albumen protein within the interstitial space. This is schematically represented in Fig. 13.4 which shows how fibrous bundles are formed after freezing. Indeed this ability to create fibrous bundles from the filamentous hyphae differentiates mycoprotein from other meat-free proteins in its ability to introduce fibrosity – a highly desirable sensory characteristic within the overall challenge to emulate the eating quality of meat. A simplistic material science definition of fibrosity in food is 'compression-fracture' happening repeatedly as

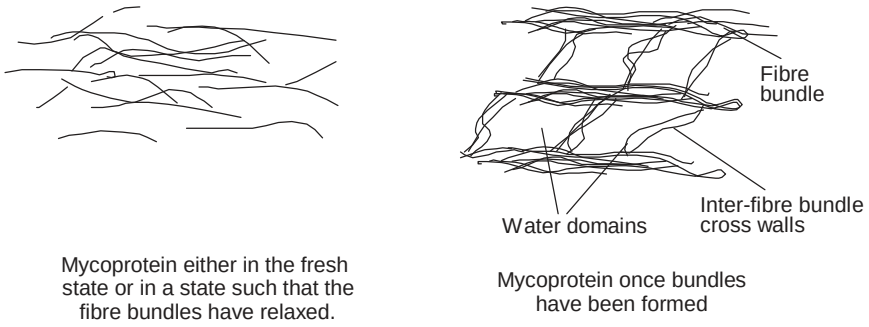


Fig. 13.4 Schematic representation of the effect of freezing on texture development with mycoprotein.

the food is chewed and on this basis it can be imagined that creating fibrous bundles with mycoprotein helps to fulfil this requirement.

The strength of the mycoprotein and egg system can be visualised as being dependent on the properties of both the entangled fibres and the gelled protein – a fibre gel composite. For example, if the rigidity of the hyphae increased but the albumen gel strength remained constant then the overall system strength would increase. Conversely, if the rigidity of the hyphae remained constant but the concentration of the albumen gel increased then the overall system would, again, become stronger as a whole.

If we assume (and only for the sake of argument) that the rigidity of the hyphae and strength of the gel are constant, then what other factors impact on overall system strength? There are some interesting hypotheses here.

13.4.1 Hyphal morphology

Hyphal aspect ratio and degree of branching will intuitively affect the degree of entanglement – if the aspect ratio were 1, for example, as with a sphere, it would be somewhat difficult to create an entangled mass.

We have also observed that below *ca*. 400 microns in length there is a tendency to increase the bittyness characteristic which is undesirable in finished product eating quality. We continue to explore the correlations of overall hyphal morphology (length and branch frequency) with eating quality. This could be argued to be somewhat academic given that producing a single 'ideal' hyphal geometry during fermentation is not yet possible.

In addition, we continue to develop our understanding of the nature of 'hyphal conformance' and its correlation with eating quality. There are several observed 'conformations of hyphae' which can be overlooked if the hyphal length is measured by following total length. For example, polymer science shows that 'tip-to-tip' measurement better help describe the physical properties of concentrated solutions or melts.

Hyphal interaction
Apart from the physical nature of hyphal entanglement there will also be a degree of interaction between the 'surface chemistry' of the hyphae and also the amount of interaction with the gelled interstitial albumen. For example, imagine pulling an individual hyphae out from the biomass. The resistance to pulling the hyphae out is determined both by the entanglement and the adhesive forces of the albumen gel (its gel strength).

Thus, the nature and scope of the entanglement and the adhesive forces created by the albumen must be correlated with eating quality but remain the topic for further research.

Orientation and dispersion of the hyphae
If the hyphae could be aligned totally then the response of the system to any applied force would depend on whether the hyphae were parallel or perpendicular to the applied force. If randomly entangled then no such effect would occur.

Thus, dispersion is important since if the hyphae are dispersed as clumps then it will be the behaviour of 'the clump' that will be most important and given that clumps tend to an aspect ratio of one then we risk a preponderance of non-entangled clumps. In addition, water trapped within the clumps becomes unavailable for solute dissolution, e.g. flavour and albumen.

Hyphal turgor
The amount of turgor that remains within the hyphae post RNA reduction must impact on eating quality either through a correlation with features such as entanglement, system strength, surface properties or by the degree of 'expressible water' that the cells possess and impact on degree of solute dissolution.

Phase volume
Hyphal properties will impact on the packing density that can be achieved. The packing fraction of a system of rigid rods will be 0.5. However, the flexible hyphae might be argued to achieve 0.6 (i.e. 60 g hyphae occupy 100 ml). But how important is this, and what is in the 'space' not occupied by the hyphae? Sub-optimal distribution of the albumen binder, for example within this matrix, will lead to binder-starved regions and product weakness.

13.4.2 Process variables that impact on quality

Thus, we have developed our arguments that the properties of the fibre gel composite are essential determinants of finished product quality and that these can be influenced by the characteristics of both the gel and also the hyphae. In addition, we know that the process variables can be powerful influences in finished product texture creation.

Mixing

From the points raised on page 342 regarding phase volume, it is clear that the degree of ingredient dispersion will have an impact on eating quality – mixing of ingredients of variable degrees of hydration characteristics into essentially a viscoelastic medium is theoretically an uncontrolled process. Imagine the challenge for the Quorn™ process of the two-tonne mix where uniform ingredient dispersion, under these conditions, down to a 10–20 g retail Quorn™ piece size is a key determinant of product quality.

And despite this challenge, the process works well, delivering finished product of reproducible quality. Nevertheless, we continue to deepen our understanding of the influence of the degree of mixedness of the albumen (or other powdered ingredients such as flavour). For example, we have proposed from our considerations on phase volume that we do not achieve uniform dispersion and thus will create different zones of albumen concentration within the mix with concomitant impact on finished product quality. We know that albumen (and other protein) gel strength is also heavily influenced by the ionic strength of the system – an inevitable consequence of under mixing. This will also impact on the degree of juiciness in the product as well as flavour release, since high albumen gel concentrations will 'lock away water' and make flavour inaccessible as the gel will act as a 'flavour sink'.

Fibre alignment

It is known that fibre alignment occurs during induced extensional flow of mycoprotein (Miri, 2004; Miri *et al*., 2005). Within the manufacturing process this begins during forming process pre-cook (Fig. 13.3). However, new technologies are currently being explored with the potential to enhance this alignment yet further which in theory develops a structure and eating quality more closely related to muscle. Figure 13.5 illustrates just how this technology is capable of increasing the packing density of the hyphae, especially when compared with the orientation shown in the raw material in Fig. 13.2. This impacts significantly on finished product sensory properties.

Heat setting

Heat setting is a key unit process. Any mixing effects and fibre alignments are 'fixed' at this point, converting a soft and dough-like texture to a rubbery precursor to meat-like eating quality.

We know that hyphae respond differently to heat setting by steaming than to heat setting by microwaving, for example. In the latter, the hyphae tend to be plumper and protein dispersion is more uniform at the surface (see page 342). We also know that albumen proteins denature at approximately three key transition endotherms between 70 and *ca*. 85 °C and that the nature of the denaturation and formation of a gel is heavily influenced by environmental conditions such as mixedness, ionic strength and rate of heating and cooling.

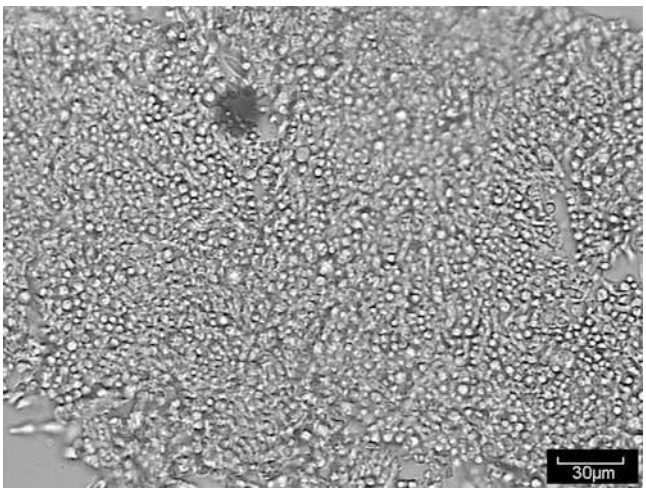


Fig. 13.5 Extensional flow and enhanced alignment of mycoprotein fibres.

We know that air inclusion within the mix can cause gas bells to form on heating, promoting areas of softer texture. This can be a particular problem if CO_2 has been used to cool and has not been adequately removed by vacuum.

Freezing and frozen storage

Of all the unit processes employed in the production of Quorn™ none is more impactful than freezing and frozen storage (Evans, 1996). We know that freezing converts the rubbery mass post steaming to a fibrous meat-like texture. We believe that approximately 50% of the texture is formed after freezing and that texture further develops over frozen storage of *ca*. 3 weeks.

We know that for optimal texture we require relatively slow freezing (compared with conventional food freezing processes) and that ice crystals appear to compress the mycoprotein fibres into bundles that convey meat-like texture in the mouth (Rodger and Angold, 1991). We also know that 'Ostwald ripening' of these crystals appears to improve 'meatiness' over time in storage but that lengthy storage can result in product defects, typically a change in the distribution of expressible water giving rise to high initial juiciness but then a dry and woody chew.

The idea that we create fibrous bundles by freezing has already been illustrated in Fig. 13.4. Figure 13.6 shows a scanning electron microscopic view of the nature of these fibrous bundles in mycoprotein after freezing. The surface of this sample has been fractured whilst frozen, so that the surface is a cross-section showing the fibrous nature of the Quorn™ (paler contrasting material) with the voids (darker regions), which would have been caused by the formation of the ice crystals.

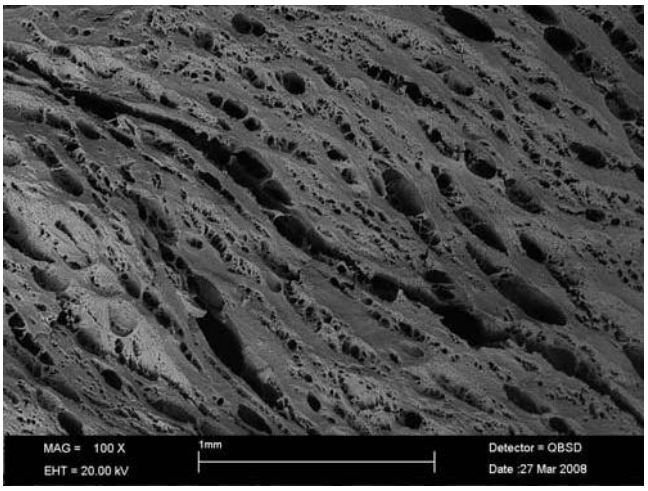


Fig. 13.6 Ice crystal growth on freezing forms highly packed hyphal domains and gives rise to fibrous structure.

13.4.3 Some surprising features of mycoprotein

Mycoprotein can also behave as a continuous phase into which previously textured mycoprotein (or other textured proteins) can be dispersed. Finnigan and Stephens (1996) have demonstrated this ability in the creation of so-called comminute textures such as burgers and sausage where the whole-muscle-like texture is not appropriate. This has allowed the commercial application of mycoprotein in creating a range of textures beyond the characteristic whole-muscle texture of Quorn™ fillets or pieces through to the more granular comminuted textures of Quorn™ burgers and sausages.

When a dispersion of mycoprotein in water at *ca*. 20% w/v is subjected to very high pressure homogenisation (in excess of 20,000 psi) we observe significant conformational change to the hyphae. The effect of this pressure is to change the aspect ratio of the hyphae and to create particles that when mixed into certain food systems have the ability to behave as fat mimetics. Finnigan and Blanchard (2009) demonstrated this effect in the creation of frozen ice creams at *ca*. 4% total fat but with a creamy mouthfeel suggestive of a more indulgent high fat system.

13.5 Nutritional properties of mycoprotein

Figure 13.7 is a further schematic representation of mycoprotein and shows again the filamentous and branched nature of the hyphae so important in the creation of meat-like textures.

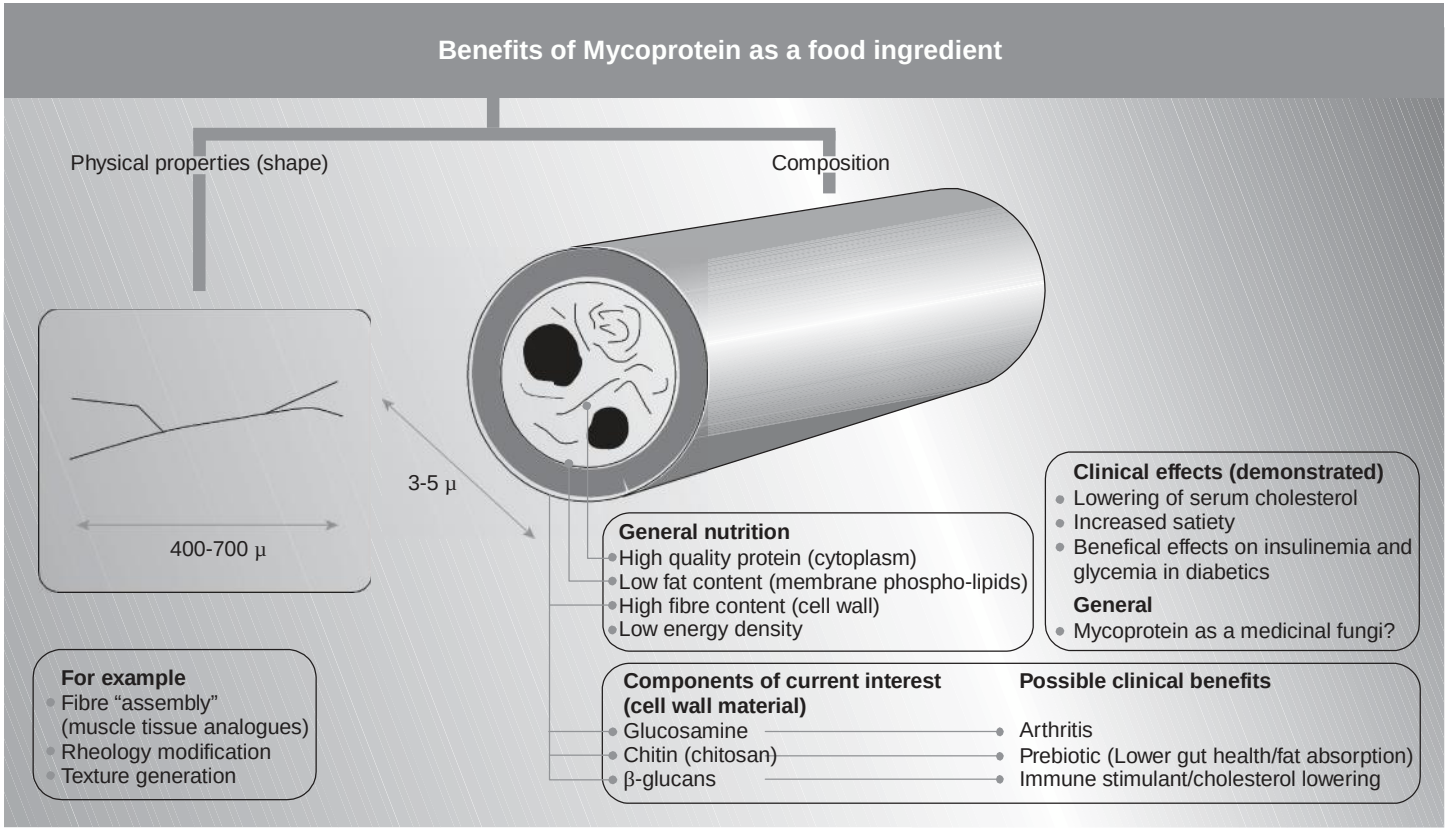


Fig. 13.7 The benefits of mycoprotein as a food ingredient.

Table 13.1 Nutritional composition of mycoprotein per 100 g (wet weight)

Nutrient	Quantity
Energy (KJ)	360
Energy (Kcal)	86
Protein (g)	11.5
Total carbohydrate (g)	1.7
of which sugars	0.8
Total fat (g)	2.9
of which saturates	0.6
of which monounsaturates	0.5
of which polyunsaturates	1.8
Dietary fibre (NSP) (g)	6.0
Sodium (mg)	4

Figure 13.7 also shows a schematic cross-section through the hyphum and depicts a level of cellular organisation typical of ascomycete fungi. Table 13.1 shows the nutritional properties of mycoprotein (Denny *et al.*, 2008). The protein content, typically 11.5 g/100 g, contains all of the essential amino acids and is of high quality. Edwards and Cummings (2009) recently reported a revision to the protein digestibility corrected amino acid score (PDCAAS) showing the score to be 0.996 – a near perfect protein.

Mycoprotein also contains a favourable fatty acid profile and is *ca.* 6% dietary fibre (Denny *et al.*, 2008). This relatively high fibre content combined with its low fat and saturated fat and excellent protein quality make mycoprotein a valuable ingredient for use in a healthy diet. In addition, the fibre is unusual in that it is predominantly polymeric n-acetyl glucosamine with beta 1,3 and 1,6 glucan. The nature of the fibre is thought to play a causal role in the clinical studies that have reported a benefit from a diet rich in mycoprotein in maintaining healthy blood lipids (Turnbull *et al.*, 1990) as well as promoting satiety (Williamson *et al.*, 2006). The clinical studies that have been carried out over the past twenty years into the beneficial effects of mycoprotein on health have been reviewed by Denny *et al.* (2008). Research is now continuing into understanding the causal mechanisms involved with satiety.

The link between diet and health has never been stronger and mycoprotein has shown demonstrable benefits in addressing so-called diseases of affluence and providing solutions to lifestyle choices in the developed world. However, with the debate increasingly moving to looking again at the global challenge of an ever-expanding population and food (or protein) security, alongside a growing awareness that food and agriculture are key contributors to greenhouse gas emissions (Garnett, 2008), is it time to look again at 'the first new food since the potato' in the context of sustainability and so fulfil the original vision of Rank in 1964?

Table 13.2 Chronology of launches of Quorn™

Country	Year of launch
United Kingdom	1985
Belgium	1992
Holland	1993
Switzerland	1996
Sweden	1998
Eire	2002
USA	2002
Denmark	2007
Norway	2008
Australia	2010

13.6 Regulatory status

From Rank's initial thoughts in 1964 to the first commercial product took over twenty years of work, much of which in the early days of the project was commercially speculative and required significant investment in order to create a robust evidence base of food safety for what was then a 'novel food'. Regulatory approval in the UK for unrestricted sale was granted in 1985 by the then Ministry of Agriculture Fisheries and Food (Solomons, 1987; Edwards, 1993). Approval was given after detailed review of the food safety data that ran to twenty-six volumes (Trinci, 1992) and described the food safety testing sequence for mycoprotein that ranged from initial *in-vitro* tests to human chronic studies. This is summarised by Miller and Dwyer (2001). Since 1985 an estimated 160,000 tonnes of mycoprotein have been produced, equivalent to approximately 3 billion meals currently on sale in ten countries worldwide (Table 13.2) in products marketed under the Quorn™ brand.

The approval for sale in 1985 was long before the novel foods regulations of 1997 (EC Regulation 258/97) and thus mycoprotein was not a novel food in the context of EU legislation.

13.7 Future trends: mycoprotein and sustainability

The challenges we face to deliver a sustainable lifestyle to a world population predicted to grow to nine billion by 2050 are enormous. Central to this issue is the notion of future food security and the sustainable diet. Some are now calling for a paradigm shift in food policy and a reduction in the consumption of animal fats and protein (Friel *et al.*, 2009).

The FAO predict that between 2001 and 2050 global meat and milk consumption will double (FAO, 2006). At present, nearly 60 billion animals

are used globally to produce meat, milk and eggs. This figure could rise to 120 billion by 2050. Such a marked upsurge could have an overwhelming impact on climate change and the environment, as well as fuelling epidemics of obesity and heart disease within the industrial nations and emerging economic powers.

The FAO 2006 report, *Livestock's Long Shadow* (FAO, 2006), recognised that animal production is a major contributor to greenhouse gas (GHG) emissions measured as embedded carbon or carbon dioxide equivalents $CO_{2(e)}$.

Livestock production accounts for 30% of the world's surface land area. This is increasing, with 70% of previously forested land in the Amazon occupied now by cattle pasture and crops for animal feed. This erosion of the rainforest has a marked effect on carbon sequestration and thus a negative impact on the carbon cycle and with 20% of pasture land degraded because of overgrazing, compaction and erosion.

The predicted doubling of global animal production by 2050 will generate huge increases in livestock-related direct and indirect land and water use as well as GHG emissions in the coming decades. Nitrous oxide emissions are projected to increase by 35–60% by 2030 due to increased manure production and use of nitrogen fertiliser with new industrial farms for pigs and poultry set to raise global emissions of methane from pig slurry and nitrous oxide from poultry manure (House of Commons report, 2009).

13.7.1 Life cycle analysis (LCA)

A life cycle analysis (LCA) of the production of Quorn™ mince relative to beef mince has recently been carried out by the Institute of Energy and Sustainable Development, De Montfort University (Allen *et al*., 2009; Finnigan *et al*., 2010).

By using the complex agricultural model developed by Williams *et al*. (2006), it is possible to carry out an analysis to estimate the global warming, acidification and eutrophication potentials associated with the agricultural aspects of both meat and Quorn™ production. Using this tool also provides a consistent approach for the production of both foods. It should be noted that the beef and chicken production data used in the model are based on UK profiles for commodity production, and the calculations do not include the production of feed that originates from overseas (such as soya bean production in South America and maize grain production in the USA). For the production of mycoprotein and its subsequent processing into Quorn™ products, only the production of carbon dioxide equivalents (CO_2) from energy and water consumption has been estimated in this initial phase. Therefore, the most telling comparison that can be made at this stage is that associated with the carbon footprint of each production. Once validated and analysed in greater detail, this would provide the

starting point for completing a carbon footprint based on PAS 2050 (BSI, 2008) designed to assess the product life cycle of greenhouse gas (GHG) emissions.

Initial LCA model conclusions

From the current available data, initial estimates suggest that tonnes of CO_2 equivalents released per tonne of product (ending at the storage of the products prior to distribution and consumption) are 14.3 t CO_{2e} per tonne of beef and 6.8 t CO_{2e} per tonne of Quorn™ mince. For Quorn™ products the production of mycoprotein contributes 3.1 t CO_{2e} and the rest is generated from the processing of the mycoprotein into Quorn™ products.

These initial estimations suggest that Quorn™ mince may have a significantly lower $CO_{2(e)}$ emission rate than the production of beef. However, it should be noted that these are estimates for the Quorn™ products due to the missing primary data. With more detailed and accurate information from suppliers it will be possible to make a more comprehensive assessment of the contribution of Quorn™ products to the release of embedded CO_2.

Recent evidence also suggest that water consumption for Quorn™ mince is *ca*. 66% of that shown for the beef, whilst convesrion of glucose to mycoprotein by fermentation suggests a land use of 0.3–0.5 ha/te mycoprotein compared with beef which is typically 5 ha/te total carcass weight (Allen *et al*., 2009).

On this basis and in conjunction with the ongoing nutritional research into satiety, mycoprotein would seem to be well placed within the current debate on food and protein security and so may yet fulfil the potential of the original 1964 vision of J Arthur Rank.

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Algal proteins

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Abstract: Some algae, particularly blue-green and green algae, contain very high levels of protein, typically 40 to 60% (of dry matter), that can be used as functional food ingredients. Algal proteins possess a high nutritional value in terms of protein content, amino acid quality and nutritional acceptability. Their functional properties, such as gelation, water and fat absorption capacity, emulsification capacity, foaming capacity, etc., are also comparable with those of terrestrial plants. Besides their natural character, other important aspects related to the algal proteins are their easy cultivation, their rapid growth and the possibility to control the production of some specific compounds by manipulating the cultivation conditions. Algal proteins possess a great economic potential for use in functional, processed foods and health foods.

Key words: algae, algal proteins, functional properties, nutritional value, functional ingredients, *Chlorella*, *Spirulina*.

14.1 Introduction

Algae are particularly attractive as natural sources of compounds that can be used as functional food ingredients. Seaweed phycocolloids (such as carrageenans, agar, alginate, etc.) are widely used in food and pharmaceutical products as gel-forming or thickening agents. Algae are also rich in lipids, minerals (e.g. calcium and iodine), vitamins, soluble dietary fibres and other bioactive molecules and help to meet human daily requirements. In recent years, further attention has been given to algal proteins to be used as alternative protein supplies as a response to the world's growing population, and thus food needs.

As shown at Table 14.1, some algae contain very high levels of protein, typically 40 to 60% (of dry matter) (Becker, 2007). The nutritive value of algal proteins is comparable, and in many cases superior, to that of most conventional protein feed supplements in term of gross protein content,

Table 14.1 General composition of different algae (% of dry matter) (from Becker, 2007)

Algae	Protein	Carbohydrate	Lipids
Anabaena cylindrica	43–56	25–30	4–7
Aphanizomenon flos-aquae	62	23	3
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	3
Euglena gracilis	39–61	14–18	14–20
Porphyridium cruentum	28–39	40–57	9–14
Scenedesmus obliquus	50–56	10–17	12–14
Spirogyra sp.	6–20	33–64	11–21
Arthrospira maxima	60–71	13–16	6–7
Spirulina platensis	46–63	8–14	4–9
Synechococcus sp.	63	15	11

unique amino acid quality and composition and nutritional acceptability (Indergaard and Minsaas, 1991; Chronakis, 2000). The gelation, water and fat absorption capacity, emulsification capacity, foaming capacity and stability of algal proteins are also comparable with those of terrestrial plants. Moreover, the great genetic diversity of algae and genetic engineering of algal proteins may be able to lead to a great variety of protein products with better yields than other protein feed supplements.

The utilisation of algal proteins in foods is relatively limited but becomes steadily more attractive as profit is recovered from algae biomass cultivation in comparison with conventional protein production. Algae are all autotrophic and, in addition to water, require only light, carbon dioxide and inorganic nutrients to sustain growth (Radmer and Parker, 1994; Wood *et al*., 1991). These inexpensive requirements, together with the high growth rates of the unicellular genera, and the wide range of growth conditions that algae can survive, make some algae a potentially attractive source of biomass and offer significant technical and commercial advantages.

14.1.1 Genetic and structural diversity of algae

Algae are chlorophyllin aquatic plants that belong to the *Thallophyta* phylum. They are a diverse group of cryptogamic plants and are morphologically found in the form of single cells, colonies of physiologically independent cells or large, complex thalli with fronds over 60 m in length (Rasmussen and Morrissey, 2007; Hallmann, 2007).

All algae are autotrophic, and none are differentiated into true roots, stems or leaves. This lack of tissue specialisation is an advantage in enabling many of the algae to grow more rapidly than conventional agricultural

crops. At the same time, however, the absence of readily observable features poses a number of problems to the taxonomist. In the present classification of algae, it will be necessary only to identify those most important families that might be considered as sources of protein for human food and/or animal feed and ones that are of potential economic importance.

There are two categories among algae: *micro-algae*, which are morphologically, or at least physiologically, unicellular, and *macro-algae*, which are macroscopic plants of marine benthoses (thalloid) and in which a degree of tissue differentiation is clearly visible (Wood *et al*., 1991; Darcy-Vrillon, 1993).

Three families of macro-algae can be distinguished according to the nature of their major pigments: (i) the *Chlorophyceae* or green seaweeds (i.e. *Ulva*, *Enteromorpha*), where the carotenes and the chlorophylls between them show maximum light absorption in the red and blue regions of the spectrum; (ii) the *Phodophyceae* or red algae (i.e. *Porphyra*, *Rhodymenia*), which are characterised by the possession of phycoerythrin, an accessory pigment in photosynthesis that shows maximum absorption of light in the green region of the spectrum; and (iii) the *Phaeophyceae* or brown algae (i.e. *Fucus*, *Laminaria*, *Ascophyllum*, *Macrocystis*), whose major pigments are chlorophyll and fucoxanthin. Brown and red algae have been employed for many years to extract various colloids (polysaccharides) that are used for their functional properties. In addition to these widely spaced classes, algae are also members of three other groups in this scheme, namely the *Plantae* (which contain several classes of the *Chlorophyta*), the *Alveolates* (including the *Dinophyceae*) and the *Stramenopiles* (which include the *Heterokont* algae) (Radmer and Parker, 1994).

Cyanobacteria, or blue-green algae (i.e. *Spirulina*, *Anabaena* and *Nostoc*), are a group of extraordinarily diverse prokaryotes that range from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensely pigmented, heterotrophic to autotrophic, psychrophilic to thermophilic and marine to freshwater.

Micro-algae represent a subset of single-cell micro-organisms that generally grow autotrophically using CO_2 as their sole carbon source and light as energy. Some species are heterotrophic, however, and can use different forms of organic carbon as sources of nutrients. There are several types of micro-algae species; these include for example the 'weed' green species of the genera *Chlorella*, *Micractinium*, *Dunaliella* and *Scenedesmus* in freshwater systems and *Phaeodactylum*, *Micractinium* and *Skeletonema* in marine systems. Micro-algae have a wide range of physiological and biochemical characteristics, many of which are rare or absent in other taxonomic groups. The two predominant characteristics of the micro-algae are their high efficiency by which they are able to convert solar energy into cellular biomass and the high proportion of the biomass that exists in the form of protein. Further classification of the algae is much more complicated but is beyond the scope of the present review.

Genetic engineering techniques (algal transgenics) that manipulate algae by molecular biology have now been under development for 30 years and it is now possible to molecularly manipulate a number of different algae to produce desired compounds (antibodies, insecticidal proteins, vaccines) that were previously not economical (Hallmann, 2007). It will be an important challenge to be able to use molecular biology to take advantage of algal products and individual protein characteristics in an economical fashion.

14.2 Cultivation and production of algae and algal proteins

Two major approaches have emerged in the design of large-scale facilities for the cultivation and production of algae and algal proteins. On one side are the sophisticated *enclosed bioreactors*, which feature close control of environmental parameters and operation conditions (Qiang and Richmond, 1994; Borowitzka, 1997; Spektorova *et al*., 1997). On the other side are low-cost *open units*, such as ponds or oblong raceways, unmixed or mixed by paddle wheels, pumps or air-lift systems (Barclay *et al*., 1994; Gladue and Maxey, 1994; Patterson, 1996).

The supply, distribution and utilisation of light in algal cultures are central aspects and receive particular attention in the design of photobioreactors (Eriksen, 2008). Light intensities are high at culture surfaces, but absorption and scattering result in lower light intensities and complex photosynthetic productivity profiles inside the cultures. High light intensities at culture surfaces may cause photoinhibition, and the efficiency of light energy conversion into biomass (photosynthetic efficiency) is low. The photosynthetic efficiency increases as light becomes limiting, but the productivity is negatively affected by central, light-deprived zones (Eriksen, 2008; Qiang and Richmond, 1996). Although photobioreactors are generally more reliable than ponds or tanks, their major disadvantages are a high capital cost of construction, a requirement of cooling systems and technical difficulties in achieving uniform illumination in vessels with a low surface area to volume ratio.

On the other hand, open systems, while economical, are technically difficult to mix, monitor and control. Axenic operation is impossible, and the maintenance of a monoculture can be difficult, limiting the use of these systems to algae that are capable of very rapid growth or algae that can tolerate extreme culture conditions that restrict the growth of foreign species, such as alkaline conditions that favour the growth of *Spirulina*. Open ponds are appropriate for producing biomass or cell constituents where construction and operation costs are major constraints. Existing commercial scale algal cultures use very large (5000 to greater than 5 000 000 m^2) open-air ponds, that can be mixed with paddle wheels.

An alternative to photobioreactors and a potential means for substantially reducing growth costs is to use heterotrophic algae and to grow them

Table 14.2 Single cell protein production from algae (from Anupama and Ravindra, 2000)

Organism used	Substrate
Caulerpa rocemosa	Carbon dioxide + sunlight
Chlorella salina CU-1(28)	Saline sewage effluent
Chlorella spp.	Carbon dioxide
Chlorella spp. (M109, M121, M122, M138, M150)	Carbonate and seven other compounds
Dunaliella	Carbon dioxide + sunlight
Chlorella & Diatoms	Carbon dioxide + sunlight
Laminaria	Carbon dioxide + sunlight
Porphyra	Carbon dioxide + sunlight
Sargassum	Carbon dioxide + sunlight
Spirulina maxima	Carbon dioxide + sunlight
Spirulina spp.	Carbon dioxide

in conventional fermentors. In this case, algae and algal proteins are cultured using glucose (or other carbon compounds) as a source of both carbon and energy as shown at Table 14.2 (Anupama and Ravindra, 2000). Systems for continuous sequential heterotrophic/autotrophic production of algae biomass composed of a conventional fermentor for the heterotrophic phase and a tubular photobioreactor for the autotrophic phase have also been constructed (Ogbonna *et al*., 1997).

Although the production of the algal protein depends on the growth phase and/or culture conditions (Morton and Bomber, 1994), the productivity of unicellular organisms is considerably higher and does not compete with terrestrial plants. The yields available via mass cultivation of microalgae generally amount to between 20 and 50 times more protein in terms of area than peak soybean yields, with an achievable production of 50–110 tons/ha/year and a potential maximum amounting to 500 tons/ha/year (Darcy-Vrillon, 1993). For instance, *Spirulina* algae can be grown in troughs in the open, and the algae can be separated by simple filtration. It has been estimated that one acre (0.4 ha) will yield 10 tons of protein as compared to 0.16 tons of wheat and 0.016 tons of beef (Borowitzka, 1995). The simplicity of S*pirulina's* cellular structure avoids extraneous activity and wasted energy, allowing rapid photosynthesis, growth and an almost total concentration in nutrient production. Large numbers of ribosomes in *Spirulina* enable it to synthesise proteins more rapidly than in other plants. In fact, *Spirulina* has a photosynthetic conversion rate of 8 to 10%, compared to only 3% in most terrestrial plants, such as soy beans.

Overall, the cultivation of algae offers several advantages over the use of conventional higher plants, and include high growth rates, high uptake and release rates promoted by a large surface to volume ratio, strains that can tolerate extreme conditions, no need for high-quality agriculture soils,

a possibility of high-density growth using closed photobioreactors with semi-controlled parameters and highly valued end-products (Rasmussen and Morrissey, 2007).

14.3 Composition of algal proteins

14.3.1 Protein content of micro-algae and some macro-algae

Table 14.3 shows the protein content of micro-algae and some macro-algae (Becker, 1986; 1988; Ito and Hori, 1989; Mabeau and Fleurence, 1993; Arasaki and Arasaki, 1983; Hayashi *et al*., 1986). There is a high level of proteins in green and blue-green algae (generally 40–65% of dry weight), and these values are comparable with the protein content of edible, land high-protein vegetables. In some red seaweeds, such as *Palmaria palmata* (dulse), *Porphyra tenera* (nori) and *Porphyra yezoensis*, proteins can

Table 14.3 The protein content of micro-algae and some macro-algae (g/100 g dry weight) (reprinted from Becker, 1986; 1988; Ito and Hori, 1989; Mabeau and Fleurence, 1993; Arasaki and Arasaki, 1983; Hayashi *et al*., 1986)

Algae	Protein
Anabaena variabilis	56.1 ± 2.2
Anabaenopsis sp. (Albufera)	52.2 ± 2.5
Chlorella vulgaris	51.0–58.0
Chlorella pyrenoidosa	57.0
Dunaliella salina	57.0
Dunaliella bioculata	49.0
Enteromorpha linza	19.3
E. compressa	12.4
Euglena gracilis	39.0–61.0
Hormidium sp.	41.0
Nostoc commune	39.9 ± 1
Nostoc muscorum	67.0
Nostoc paludosum	40.4 ± 4.5
Nostoc sp. *(Doñana)*	51.6 ± 4.6
Porphyra tenera	27.5
Prymnesium parvum	28.0–45.0
Scenedesmus obliquus	50.0–56.0
Scenedesmus quadricauda	47.0
Stigeoclonium sp.	51.0
Synechococcus sp.	63.0
Spirulina maxima	60.0–71.0
Spirulina platensis	46.0–62.0
Ultotbrix sp.	45.0
Ulva sp.	15.0–25.0
Uronema gigas	58.0

represent up to 25%, 30–47% and 45% of the dry matter, respectively (Darcy-Vrillon, 1993; Fujiwara-Arasaki *et al*., 1984). Except for *Undaria pinnatifida*, whose protein content is 11–24%, the other brown algae (i.e. *Alaria esculenta*, *Ascophyllum nodosum*, *Fucus veicolosus*, *Laminaria digitata*, *Himanthalia elongata* and *Hizikia fusiforme)* have a rather low protein content (5–15% of the dry weight).

14.3.2 Amino acid composition of algal proteins

Selected data on the amino acid profile of various micro-algae are given in Table 14.4 and compared with some basic conventional food items and a reference pattern of a well-balanced protein recommended by WHO/FAO. It can be seen that the amino acid pattern of almost all algae compares favourably with that of the reference protein and the other food proteins (Becker, 2007).

The edible algae generally exhibit similar amino acid patterns (Fujiwara-Arasaki *et al*., 1984). Algae proteins have a high content of the essential amino acids valine, leucine, lysine and phenylalanine. Most algae proteins are generally deficient in the sulphur-containing amino acids cystine and methionine (Becker, 1986; 1988). The free amino acid fraction of algae is mainly composed of alanine, aminobutyric acid, taurine, ornithine, citrulline and hydroxyproline (Arasaki and Arasaki, 1983). As far as non-essential amino acids are concerned, some algae (*Undaria pinnatifida*, *Porphyra* spp., *Gracilaria* spp., *Ulva* spp.) have a high arginine content. In addition, some algae contain unusual amino acids such as chondrine, gigartine, l-baikiaine, rhodoic acid or laminine, whose physiological functions are largely unknown (Ito and Hori, 1989). The macro-algae also often contain high levels of aspartic and glutamic acid, as well as alanine. On the whole, the average essential amino acid composition of algae proteins compares favourably with that of food vegetables (Indergaard and Minsaas, 1991; Morgan *et al*., 1980). Furthermore, the essential amino acid profile shows a slight lysine deficiency in *Porphyra* spp. and a larger sulphur amino acid deficiency in *Porphyra* spp. and *Ulva* spp. Japanese *Porphyra* proteins are also rich in glycine, which would account for their distinctive flavour (Nisizawa *et al*., 1987). *Porphyra tenera* exhibits an amino acid composition close to that of ovalbumin (Table 14.5) (Mabeau and Fleurence, 1993; Arasaki and Arasaki, 1983; Fujiwara-Arasaki *et al*., 1984).

A comparative analysis of the amino acid pattern of nine algae strains (*Spirulina platensis*, *Aenbaena cylindrica*, *Calothrix* sp., *Tolypothrix tenuis*, *Nostoc commune*, *Scenedesmus acutus strains 8M* and *8L*, *Scenedesmus obtusiusculus* and *Chlorella vulgaris*) grown in outdoor mass culture in identical conditions showed an appreciable variability among different species and strains belonging to the same species (Paoletti *et al*., 1973). This observation emphasises the importance of the biochemical selection of algae strains for improving the nutritive value of the protein fraction of the

Table 14.4 Amino acid profile of different algae as compared with conventional protein sources and the WHO/FAO reference pattern (g per 100 g protein) (reprinted from Becker, 2007)

Source	Ile	Leu	Val	Lys	Phe	Tyr	Met	Cys	Try	Thr	Ala	Arg	Asp	Glu	Gly	His	Pro	Ser
WHO/FAO	4.0	7.0	5.0	5.5					1.0									
Egg	6.6	8.8	7.2	5.3	5.8	4.2	3.2	2.3	1.7	5.0	–	6.2	11.0	12.6	4.2	2.4	4.2	6.9
Soybean	5.3	7.7	5.3	6.4	5.0	3.7	1.3	1.9	1.4	4.0	5.0	7.4	1.3	19.0	4.5	2.6	5.3	5.8
Chlorella vulgaris	3.8	8.8	5.5	8.4	5.0	3.4	2.2	1.4	2.1	4.8	7.9	6.4	9.0	11.6	5.8	2.0	4.8	4.1
Dunaliella bardawil	4.1	11.0	5.8	7.0	5.8	3.7	2.3	1.2	0.7	5.4	7.3	7.3	10.4	12.7	5.5	1.8	3.3	4.6
Scenedesmus obliquus	3.6	7.3	6.0	5.6	4.8	3.2	1.5	0.6	0.3	5.1	9.0	7.1	8.4	10.7	7.1	2.1	3.9	3.8
Arthrospira maxima	6.0	8.0	6.5	4.6	4.9	3.9	1.4	0.4	1.4	4.6	6.8	6.5	8.6	12.6	4.8	1.8	3.9	4.2
Spirulina platensis	6.7	9.8	7.1	4.8	5.3	5.3	2.5	0.9	0.3	6.2	9.5	7.3	11.8	10.3	5.7	2.2	4.2	5.1
Aphanizomenon sp.	2.9	5.2	3.2	3.5	2.5	–	0.7	0.2	0.7	3.3	4.7	3.8	4.7	7.8	2.9	0.9	2.9	2.9

Table 14.5 Amino acid composition of *Porphyra tenera* protein: comparison with ovalbumin. Composition (g amino acid/100 g protein) (reprinted from Mabeau and Fleurence, 1993; Arasaki and Arasaki, 1983; Fujiwara-Arasaki *et al*., 1984)

Amino acid	*Porphyra tenera* protein	Ovalbumin
Tryptophan	1.3	1.0
Lysine	4.5	7.7
Histidine	1.4	4.1
NH_3	5.1	5.3
Arginine	16.4	11.7
Aspartic acid	7.0	6.2
Threonine	4.0	3.0
Serine	2.9	6.8
Glutamic acid	7.2	9.9
Proline	6.4	2.8
Glycine	7.2	3.4
Alanine	7.4	6.7
Cystine	0.3	1.4
Valine	6.4	5.4
Methionine	1.7	1.3
Isoleucine	4.0	4.8
Leucine	8.7	6.2
Tyrosine	2.4	1.8
Phenylalanine	3.9	4.1
Total	98.2	95.4

biomasses. All strains examined had a high content of the following essential amino acids: threonine, leucine, phenylalanine, tyrosine and valine.

The protein content of the micro-algae can be manipulated or influenced by such factors as nitrogen supply, light intensity and quality, mineral concentration, climate and the age of the cells (Wood *et al*., 1991). The variations in protein content in one and the same species grown in different locations are also very remarkable. Since protein is predominantly nitrogenous in composition, the maximum protein content and growth rates are proportional to the availability of nitrogen. Protein content also tends to be inversely proportional to carbohydrate levels and indirectly to the nitrogen level, with low levels of nitrogen giving rise to higher carbohydrate synthesis.

The quantitative ranges of proteins, as for various other components of algae, are caused by seasonal variations and may also vary with tissue of varying age and type, harvesting location, pre-processing treatment and analytical methods (Indergaard and Minsaas, 1991). It is common practice to determine the crude protein content of algae by multiplying the total nitrogen content by a conversion factor of 6.25. However, since substantial parts of the total nitrogen originate from non-protein nitrogen (nucleic acids, amines, etc.), this calculation overestimates the actual protein content

(Becker, 1986; 1988; Ryu *et al*., 1982). Non-protein nitrogen, present in part as nucleotides, can reach almost one-third of the total nitrogen in some species (*Ulva pertusa*, *Eisenia bicyclis*) (Ito and Hori, 1989). Ryu *et al*. (1982) proposed the factor method: first multiplying the quantity of each amino acid by its molecular nitrogen factor, then summing the weighted nitrogen values to provide an amino acid nitrogen content based on amino acid composition and, finally, dividing the total amino acid content by this total amino acid nitrogen value to obtain a nitrogen conversion factor.

14.3.3 Algal protein-pigment complexes

The algal protein components can be found in both a free and a pigment bound state. The pigment-protein components are supramolecular complexes in which the pigment is tightly bound to the algal protein. The action of denaturing agents makes possible a dissociation of the high-molecular-weight pigment-protein particles with the formation of smaller particles. Many algal photosynthetic pigments have been well characterised and a number of them are utilised for commercial applications. The most widely used are the phycobiliproteins, especially in immunodiagnostics and similar assays (Glazer, 1994; Zoha *et al*., 1998; Apt and Behrens, 1999).

Phycobiliproteins are water soluble, coloured, highly fluorescent compounds consisting of prosthetic groups (the bilin chromophore) covalently attached to the protein (Patterson, 1996). They are red or blue pigments that function as a photosynthetic antennae complex and optimise the photosynthetic efficiency of three types of algae: the *Rhodophyta*, the *Cyanophyta* and the *Cyptophyta*. Phycobiliproteins absorb light in the visible region of about 450–650 nm and transfer it through an energy chain from the high-energy red phycoerythrin to the lower-energy blue phycocyanin to the light blue allophycocyanin to chlorophyll α and finally to the
photosynthetic reaction centre (Arad and Yaron, 1992). Phycoerythrin is located at the periphery of the phycobilisomes; phycocyanin is located between phycoerythrin and allophycocyanin (McColl and Guardfriar, 1987). Phycocyanin and phycoerythrin are proteinaceous in structure and exhibit a high extinction coefficient and fluorescence. They can easily be coupled to proteins (monoclonal antibodies, avidin and treptavidin) or to small molecules (biotin and digoxigenin) with little alteration in the spectroscopic properties of the chromophore (Patterson, 1996; Rattray, 1989).

Phycobiliproteins can constitute a major proportion of algal cell protein. Growth conditions affect both the content and composition of the phycobiliproteins, while light conditions affect the ratio of various phycobiliproteins. Lower cell phycobiliprotein concentrations and lower growth rates were observed when algae were grown under high light intensity. Gantt and Lipschultz reported that the phycobiliproteins accounted for 50% of the total cell protein in *P. cruentum* (Gantt and Lipschultz, 1974). In the wild-type red macro-algae *Gracilaria tikvahiae*, phycoerythrin accounted for up

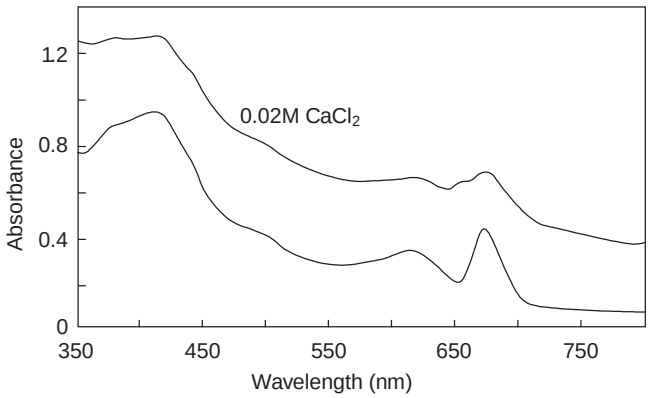


Fig. 14.1 Absorbance spectrum of ≈0.5 mg/ml *Spirulina platensis* strain Pacifica protein isolate at 25 °C, in Tris 0.1 M HCl buffer pH 7 and in 0.02 M $CaCl_2 \times 2H_2O$ in Tris 0.1 M HCl buffer pH 7 (reprinted from Chronakis, 2001).

to 10% of the soluble cell protein content (0.5% of the cell dry weight). In a number of red micro-algae the content of phycobiliproteins can be up to 30% of the total cell protein (5–10% of the dry biomass) (McColl and Guardfriar, 1987).

The pigments of the commercially important *Spirulina* algae belong to three classes: (i) chlorophyll *a*, comprising 1.7% of the organic cell weight; (ii) carotenoids and xanthophylls, which comprise approximately 0.5% of the organic weight; and (iii) two phycobiliproteins, c-phycocyanin and allophycocyanin, which normally comprise about 20% of cellular protein and are quantitatively the dominant pigments in *Spirulina* (Richmond, 1987). Figure 14.1 shows the absorption spectrum of the *Spirulina* protein in the visible region (Chronakis, 2001). The maximum absorbance at ~420 nm is due to the presence of chlorophyll-protein pigment, while the maxima at 620 and 675 nm originate from the c-phycocyanin and allophycocyanin protein pigments, respectively. Divalent ions (0.02 M $CaCl_2$) have been shown to increase the turbidity of the *Spirulina* protein solution and the absorbance, but did not significantly modify the environment of the protein-pigment complexes.

14.4 Extraction procedures and processing of algal proteins

Algal proteins can be extracted from algae in a simple way and with reasonably high yields using *classical solvent* and/or *enzymatic methods*. Classical methods use dissolution of the algae under reductive conditions in alkali. Soluble protein can then easily be separated from carbohydrates by acidic

precipitation at low pH. The extraction of algae proteins by classical procedures is hindered by the presence of large amounts of cell wall mucilage; ionic or neutral polysaccharides can also limit the efficiency of the protein extraction and the purification of proteins (Ito and Hori, 1989; Jordan and Vilter, 1991; Fleurence *et al*., 1995a; Olaizola, 2003). The cleavage or limitation of linkages between polysaccharides and proteins appear to be a determining factor for improving the extraction.

A simple procedure used for the extraction of proteins from the cells of *Spirulina platensis* strain Pacifica algae is described in Chronakis (2000). More *Spirulina protein* can be dissolved with strong alkali, and probably even more substance can be extracted with more extensive NaOH treatment. Strong alkali affects the cell content, however, and might cause a breakdown of proteins and other valuable cell components (Hedenskog and Hofsten, 1970). Disintegration followed by extraction with water or weak alkali is preferable when a high yield of unaffected cell content is desired. Decolouration of dry *Spirulina* protein may be obtained in an extraction with ethanol and acetone, yielding a pale-yellow meal with 80% efficiency.

Proteins have been extracted from the edible seaweeds *Ulva rigida* and *Ulva rotundata*. Procedures using NaOH under reductive conditions or a two-phase set (poly-ethylene-glycol/K_2CO_3) have been reported to produce the best protein yields (Fleurence *et al*., 1995a). The extraction in basic medium with NaOH and mercaptoethanol allowed an optimal recovery of protein from *Ulva rotundata* and *Ulva rigida*. However, the denaturation effect of NaOH and mercaptoethanol on the tertiary structure of proteins suggested a limited and controlled use of this procedure. It was reported that an important part of the nitrogenous components was not extracted from the green algae *Ulva* sp. using the classical method. Indeed, high contents of residual protein (20%) were observed in the insoluble fibre fraction from *Ulva* sp. (Serot *et al*., 1994). This could mean that they were either not soluble in alkaline solutions or were bound to the insoluble polysaccharide fraction.

The extractive enzymatic methods that use enzyme cellulase appear to be of little interest in terms of increasing protein extraction yields of *Ulva rigida agardh* and *Ulva rotundata* proteins. The weak accessibility of the substrates in the intact cell wall may explain these experimental data (Fleurence *et al*., 1995a). The partial improvement of protein yield after the use of a polysaccharidase mixture (β -glucanae, hemicellulase, cellulase)
also confirms this hypothesis.

The use of a polysaccharidase mixture (κ -carrageenase, β -agarase, xyla- nase, cellulase) is claimed to improve protein extractability from three certain rhodophytes (Fleurence *et al*., 1995b). The main cell wall polysac- charide (carrageenan for *Chondrus crispus*, agar for *Gracilaria verrucosa* and xylan for *Palmaria palmata*) is degraded by these hydrolytic enzymes, and protein extraction can be improved. With the exception of *Palmaria*

palmata, the highest protein yields were observed with the procedures using cellulase coupled with carrageenase or agarase for an incubation period limited to two hours. The *Chondruss crispus*/carrageenase and cellulase and the *Gracilaria verrucosa*/agarase and cellulase systems gave ten-fold and three-fold improvements, respectively, in protein extraction yield as compared to the enzyme-free blank procedure. The combined action of xylanase and cellulase on protein extraction from *Palmaria palmatadoes* did not significantly improve the protein yield. The best overall protein yield in *P. palmata* was in a *P. palmata*/xylanase mixture with a 14-hour incubation period. Cellulase also improves extractability in the cases of *Chondrus crispus* and *Palmaria palmata*; coupling cellulase with carrageenase or agarase gives optimal yields in *Chondrus crispus* and *Gracilaria verrucosa*, respectively. The enzymatic extraction procedure in these algae was milder than classical methods using NaOH and appears to limit unwanted protein-polysaccharide interactions.

A study by Arai *et al*. (1976) attempted to improve the quality of the proteins from the blue-green algal *Spirulina maxima* by peptic hydrolysis followed by plastein synthesis with papain. This process was effective in removing some photosynthetic pigments and flavour originating from the raw materials. The latter process was successful in incorporating limited amounts of methionine, lysine and tryptophan and thus in synthesising plasteins whose essential amino acid pattern resembles the pattern suggested by the FAO/WHO. These plasteins had no colour and no flavour.

Procedures described in the literature are further concerned with the extraction of particular proteins, such as proteases, peroxidases, carboxylases and phycobiliproteins (Sheffield *et al*., 1993; Zhang and Chen, 1999; Sarada *et al*., 1999). Extraction of phycobiliproteins from cyanobacteria is notoriously difficult because of the extremely resistant cell wall and the small size of the bacteria (Wyman, 1992). Various methods can be employed for extraction and purification of phycobiliproteins, but no standard technique exists for quantitatively extracting pigments from micro-algae (Jeffrey and Mantoura, 1997; Wiltshire *et al*., 2000; Ranjitha and Kaushik, 2005). There are several different physical and chemical cell disruption and protein extraction methods. Sonication in an ultrasound water bath is a very easy way to promote cell breakage and has often been used with *Phorphyridium cruentum* and *Synechococcus* (Bermejo *et al*., 2002; Vernet *et al*., 1990). To further aid the disruption process, sonication with sand, mainly small particle silica, can be advantageous (Wiltshire *et al*., 2000). Cell disruption by French press relies on blunt force to treat the samples as they are squeezed through a small orifice by the press, which disrupts the cells. Repeated freezing–thawing cycles of the samples in liquid nitrogen can aid the cell disruption process. Grinding the sample in a tissue grinder will also result in cell breakage (Stewart and Farmer, 1984). In some cases, it might be beneficial to first freeze the sample in liquid nitrogen and to grind it frozen. These techniques are a classical part of the extraction process for cyanobacteria.

Nitrogen cavitation is a gentle method of cell disruption that has not been used as much as the other techniques for extracting phycobiliproteins (Viskari and Colyer, 2003). Expanded bed adsorption has also been used to purify phycobiliproteins from *S. platensis*. The main advantage claimed by authors was the high yield achieved using this method in the steps of product extraction (crushing cells by osmotic shock) and adsorption, being able to reduce both processing time and cost (Bermejo *et al*., 2006).

Finally, not all factors that can influence the extraction procedure have yet been fully tested. The extraction of proteins should be carried out in algae collected during other periods of their biological cycle to evaluate the effect of these parameters on the amount and molecular distribution of extracted proteins. Various parameters such as the duration of the extraction, the amount of dry algae and reductive concentrations must be further improved. An optimisation of protein extraction for use in foods requires knowledge of the degradation process that usually accompanies the purification procedures and of the basic molecular features of the particular protein (Melfi *et al*., 1997). Further attempts to improve nutritional qualities and the acceptability of aquatic algae proteins varying the extraction procedures are therefore necessary.

Moreover, several processes for producing *algae protein concentrates* have been developed to the pilot stage (Milner, 1951; Enebo, 1969). Developments in downstream processing (harvesting, drying, product extraction, purification and storage) are important in terms of reducing production costs and ensuring profitability. Micro-algal biomass can be dehydrated in spray dryers, drum dryers, freeze dryers and sun dryers. In some cases the biomass may not need to be dehydrated, and extraction and fractionation can be carried out in the wet biomass (e.g. biliproteins). Further downstream processing may be needed to isolate the active compound, depending on the intended final product (Olaizola, 2003).

The effect of packaging and storage on the crossflow-dried cyanobacterium *Spirulina platensis* was also studied (Kumar *et al*., 1995). Determinations were also made of the shelf-life of *Spirulina* algae with respect to changes in chemical constituents and moisture during storage. The chemical constituents, protein and fat were less dependent on storage conditions and packaging materials, while ingredients such as phycocyanin, allophycocyanin and carotene were prone to greater loss. Materials such as a laminate of metallised polyester plus low-density polyethylene gave a longer shelf-life.

14.5 Functional properties of algal proteins

Studies on the functional properties of algal proteins are limited and have primarily been concerned with *Spirulina* and *Chlorella* proteins because of their well-known overall nutritional qualities and high protein content.

14.5.1 Spirulina

Spirulina has been produced commercially for 30 years for food and specialty feeds. *Spirulina* is 60–70% protein by weight and is a rich source of vitamins, especially vitamin B12 and provitamin A (β -carotene), and minerals, particularly iron. One of the few sources of dietary gamma-linolenic acid (GLA), it also contains a host of other phytochemicals that have potential health benefits (Belay, 2002).

The *solubility* of a protein is dependent on pH, salt, buffer ion, ionic strength, the pre-treatment received and temperature. An additional factor in algal proteins is protein-pigment complexes. In the presence of NaCl, the solubility of the *Spirulina* protein in water, unlike that of the other proteins, has been observed to decrease (Anusuya *et al*., 1981). This is most probably due to the presence of the pigment-protein complexes in the algae. As the proteins are already in the form of complexes, the force of attraction between the protein ion and the salt ion is probably reduced, accounting for its low solubility.

Thermal characteristics of Spirulina platensis

Proteins isolated from *Spirulina* have been reported to be quite complex biomacromolecules, likely to be protein and/or protein-pigment (phycocyanin) complexes rather than individual protein molecules. *Spirulina* denaturation and gel formation is therefore a complex phenomenon (Chronakis, 2001).

Studies by Topchishvili and co-workers used differential scanning microcalorimetry to investigate the thermal characteristics of iodised and non-iodised *Spirulina pl. cells* in a wide temperature range (5–140°C) (Topchishvili *et al*., 2002). It was shown that there are eight stages of transition in the heating process of *Spirulina platensis* cells in the temperature range of 5–140°C. The first stage covers the temperature range of 5–53°C, with a maximum at 45°C. It was shown that endotherm at 66°C belongs to the denaturation of *C*-phycocyanin. The endotherms with a *T*d equal to 58 and 88°C are connected with the denaturation of phycobilisome proteins, and endotherm with a *T*d of 48°C with the denaturation of protein, which is apparently connected with cell respiration.

Other studies by Chronakis (2001) and Chronakis and Sanchez (1998) have shown that the solubility profile of *Spirulina platensis* strain Pacifica protein introduced by changes in pH affects the denaturation state of the protein. Two main endothermic peaks were observed at a denaturation temperature of ≈ 67 and ≈ 109°C in 0.1 M Tris HCl buffer at pH 7, as shown in Fig.

14.2a. At a pH of 4.5 in the same buffer, almost no differences were observed for the midpoint transition temperatures while the enthalpy decreased, probably due to protein aggregation as the solubility decreased. The thermal denaturation temperature decreased by almost 7 degrees at alkaline pH.

When salt (0.004 M or 0.02 M $CaCl_2$) was added, both peak transition midpoint temperatures followed the same dependence on pH as without

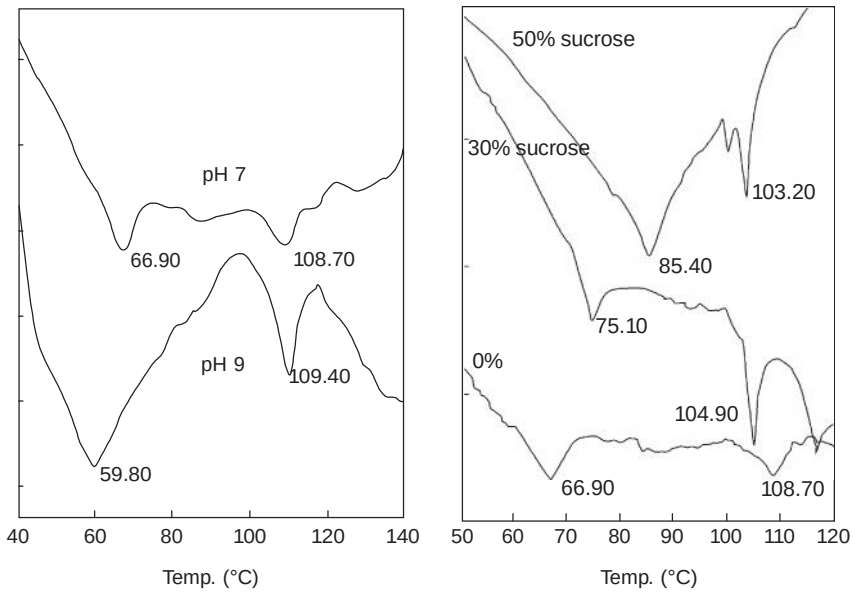


Fig. 14.2 DSC thermograms of the denaturation of *Spirulina platensis* strain Pacifica protein isolate: (a) in 0.1 M Tris-HCl buffer; heating rate was 10 °C/min. Scale is in 0.05 mW; (b) in 0.1 M Tris-HCl buffer, pH 7, and at 0, 30, and 50 wt% of sucrose, heating rate was 10 °C/min. Scale is in 0.1 mW (reprinted from Chronakis, 2001).

salt, although they progressively increased. At pH 9, the denaturation transition changes were those that were most influenced by the addition of $CaCl_2$ (62 and 63°C, respectively). Salt thus stabilises the quaternary structure from dissociation and denaturation and shifts the transitions to higher temperatures (Chronakis, 2001).

It is known that polyhydric cosolvents such as *sugars* stabilise the structure of the protein against denaturation and strengthen the hydrophobic interactions. As shown in Fig. 14.2b, the magnitude of the stabilising effect varies progressively with the amount of sucrose added (Chronakis, 2001; Chronakis and Sanchez, 1998). The first transitions were shifted to higher temperatures (from 66.9 to 75.1 and 85.9°C, with 0, 30 and 50% w/w sucrose, respectively). Nevertheless, the second transition temperatures unexpectedly decreased. It is probable that the second transition is a consequence of the reaction between protein and a reducing sugar (Maillard reaction). Thus, a significant effect of the heating rate on the midpoint temperature of the second transition could explain the effect of sugars at high temperatures.

Fluorescence and hydrophobicity of the Spirulina platensis *protein*

The intrinsic fluorescence spectra of native *Spirulina* protein isolate are shown in Fig. 14.3a. The fluorescence behaviour is similar at 25°C of

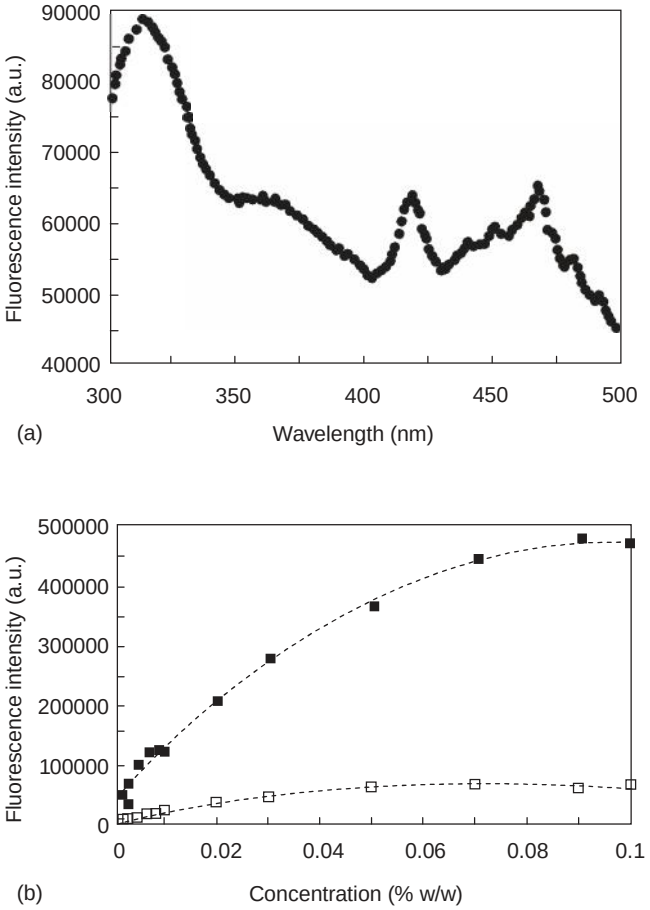


Fig. 14.3 (a) Fluorescence spectra at 25 °C of 0.05% w/w *Spirulina* protein isolate at 10 mM Na-phosphate buffer, 50 mM NaCl, pH 7.5. The λ excitation was at 280 nm and the λ emission was measured from 300 to 500 nm. (b) Fluorescence emission maxima at 420 nm as a function of *Spirulina* protein isolate concentration at 10 mM Na-phosphate buffer, 50 mM NaCl, pH 7.5. At 25 °C ( ), and at 25 °C after 3 min heating at 90 °C ( ) (reprinted from Chronakis, 2000).

denatured protein solution (heated at 90°C for 3 minutes). Thus, the spectra showed no shift of the emission maximum wavelengths but did show a modest increase in emission intensity, denoting changes in the accessibility of hydrophobic sites exposed to solvent (Chronakis, 2000).

The differences in the fluorescence emission wavelength maximum of native and denatured *Spirulina* protein-pigment complexes at ≈ 420 nm can be seen as an index of protein hydrophobicity (Fig. 14.3b). Although many

hydrophobic residues are buried in the interior of most native *Spirulina* protein, some hydrophobic groups remain exposed at the molecular surface. The increase in emission intensity following denaturation gives evidence of an increased surface hydrophobicity due to conformational changes where the protein residues are in a comparatively aqueous environment in the denatured state. Consequently, the hydrophobic residues that are exposed as denaturation proceeds could be involved in hydrophobic interactions. This has a great effect on the solubility and heat stability properties and affects the type of molecular interactions that occur during heat-induced gelation of *Spirulina* protein isolate, as discussed below.

Viscoelastic properties of the Spirulina platensis *protein*

The viscosity of *Spirulina* protein isolate decreases when the temperature increases (Fig. 14.4), as observed in most proteins. The decrease in the viscosity of *Spirulina* protein at temperatures of 10 to 50°C, follow an Arrhenius type of dependence (Chronakis, 2001). Above 60°C, the viscosity increase is closely related to the dissociation-denaturation process. Lower viscosities have been observed for protein solutions dissolved at pH 9 as a result of increased protein solubility. At a pH of approximately 5, closer to the isoelectric point of ≈3.5, the viscosity was seen to be higher than at neutral pH (no results shown). This is because the solubility decreased, as the *Spirulina* protein tends to form aggregates, which include the core that is not accessible for maximum hydration. The changes in viscosity at such conditions related mainly to the changes (increase) in particle size and are obviously of practical importance to the stability and processing of the *Spirulina* protein dispersions.

Solutions of *Spirulina* protein isolate form elastic gels during heating to 90°C. Subsequent cooling at ambient temperatures causes a further pronounced increase in the elastic moduli and network elasticity (Fig. 14.5). *Spirulina* protein isolate has good gelling properties with fairly low minimum critical gelling concentrations.

The critical gelling concentration (Fig. 14.6) of heated and cooled protein isolate preparations (about 80% protein content) is in the order of 1.5% and 2.5%w/w in buffer solution and $CaCl_2$, respectively, which are values that are fairly low for the thermal gelation of proteins (Chronakis, 2001). The difference between buffer solution and $CaCl_2$ may arise from the lower solubility of the algal protein in the presence of salt.

Studies have also been made of the molecular forces of thermal association and gelation of *Spirulina* protein (Chronakis, 2001). Hydrophobic interactions contribute substantially to the facilitation of molecular association during gelation and to the stabilisation of the gel structure in *Spirulina* protein. Hydrogen bonds reinforce the rigidity of the network of the protein on cooling and further stabilise the structure of *Spirulina* protein gels but are not alone sufficient to form a network structure. Intermolecular sulfhydryl and disulfide bonds have been found to play a minor role in the

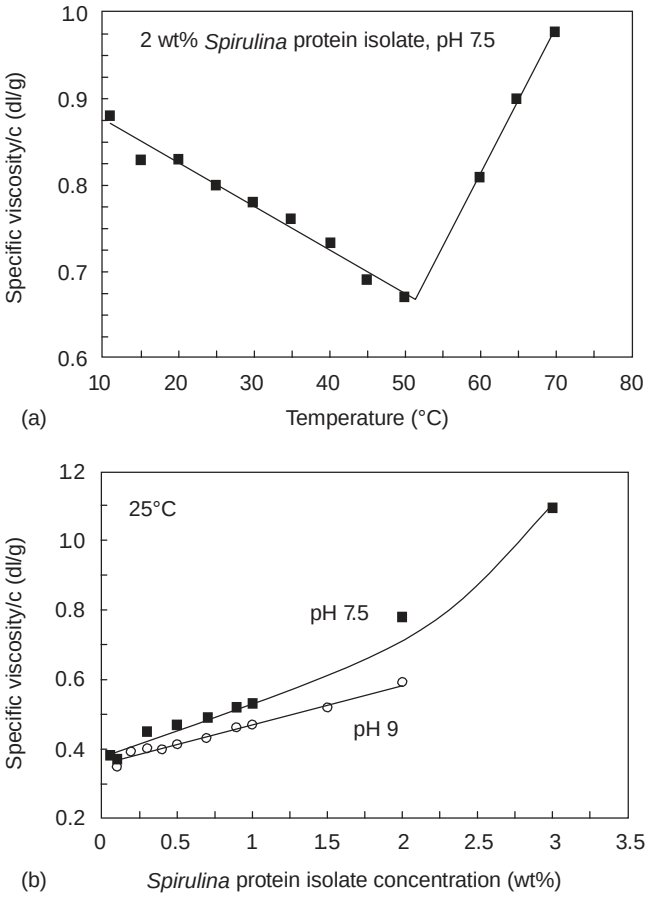


Fig. 14.4 (a) Changes in the specific viscosity of *Spirulina platensis* strain Pacifica protein isolate with temperature. 2 wt% *Spirulina* protein isolate in 0.01 M sodium-phosphate buffer, 0.05 M NaCl, pH 7.5. (b) Changes in the specific viscosity as a function of *Spirulina platensis* strain Pacifica protein isolate concentration at pH 7.5 and 9 in 0.01 M sodium phosphate buffer, 50 mM NaCl (reprinted from Chronakis, 2001).

network strength of *Spirulina* protein gels but affect the elasticity of the structures formed. Both time and temperature at isothermal heat-induced gelation at 40–80°C substantially affect network formation and the development of the elastic modulus of *Spirulina* protein gels. This is also attributed to the strong temperature dependence of hydrophobic interactions. It is likely that the aggregation, denaturation and gelation properties of *Spirulina* algal protein isolate are controlled by protein-protein complexes rather than individual protein molecules.

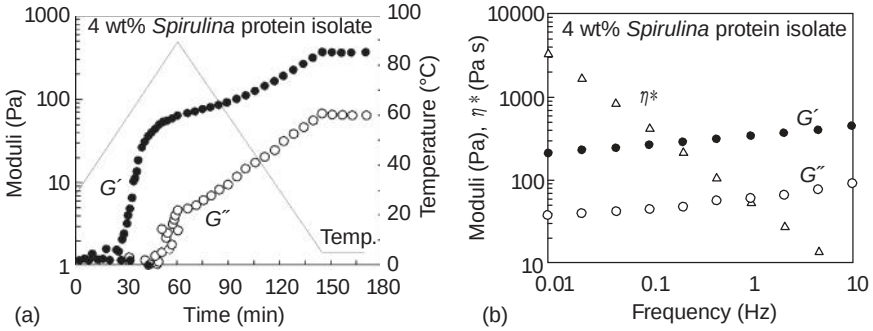


Fig. 14.5 (a) Changes in G' (•) and G'' ( ) during heating of a 4 wt% solution of *Spirulina platensis* strain Pacifica protein isolate from 30 to 90 °C and then to 5 °C at a rate of 1 °C/min. Dotted line represents the temperature history (1 Hz, 2% strain). (b) Changes in G' (•), G'' ( ), and complex viscosity η^* ( ) (D) as a function of frequency of oscillation of a 4 wt% solution of *S. platensis* strain Pacifica protein isolate at 5 °C (2% strain) (reprinted from Chronakis, 2001).

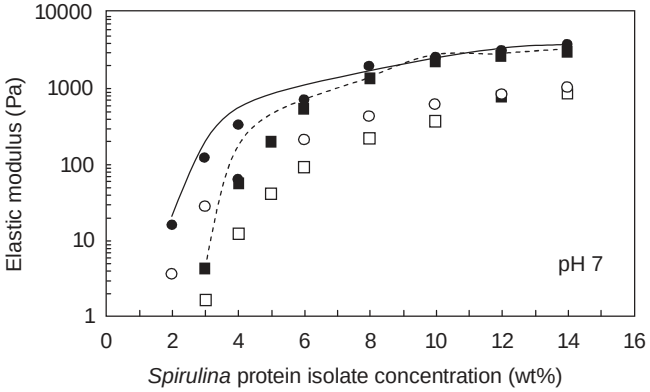


Fig. 14.6 Concentration dependence of elastic moduli for *Spirulina platensis* strain Pacifica protein isolate in 0.1 M Tris-HCl buffer pH 7 at 90 °C (o) and at 5 °C (•); and in 0.02 M $CaCl_2 \cdot 2H_2O$ in 0.1 M Tris-HCl buffer pH 7 at 90 °C ( ) and 5 °C ( ) (reprinted from Chronakis, 2001).

Surface activity of Spirulina platensis *protein preparations at air/water interface*

The surface tension of a protein sample isolated from the *Spirulina platensis* strain Pacifica was studied using the Wilhelmy plate method (Chronakis *et al.*, 2000). The protein is capable of reducing the interfacial tension at the aqueous/air interface at relatively lower bulk concentrations as compared to common food proteins (Fig. 14.7). The surface tension of the protein

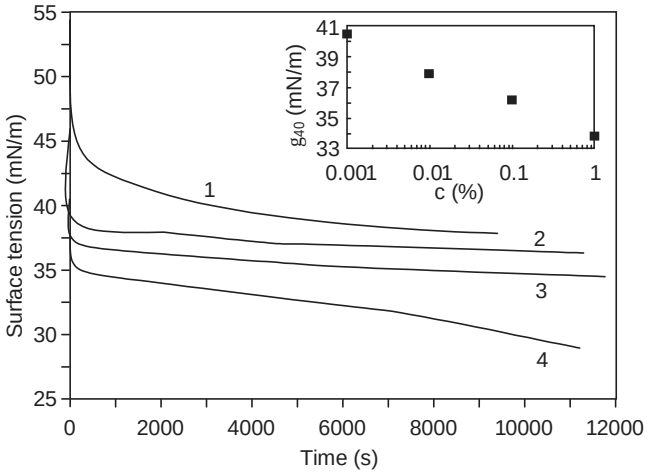


Fig. 14.7 Time-dependence of interfacial tension at concentrations of 0.001 (1), 0.01 (2), 0.1 (3) and 1% w/w (4) of *Spirulina platensis* strain Pacifica protein isolate. Inset: Surface tension after 40 minutes as a function of the bulk concentration. 10 mM Na-phosphate buffer, 50 mM NaCl, pH 7.5 was used as solvent (reprinted from Chronakis *et al*., 2000).

preparation seems to be quite independent of pH, which indicates that electrostatic interactions are of minor importance for the interfacial behaviour. It was also possible to separate out fractions with different interfacial properties by centrifugation. When the protein was spread at the air/aqueous interface, it was seen that the pressure area isotherm somewhat resembles those recorded for lipids, with a higher collapse pressure than is usually observed for proteins (Fig. 14.8). The interfacial behaviour of extracted lipids confirms that remaining traces of lipids in protein powder have only a minor influence on the surface activity of *Spirulina* protein. The surface active components are likely to be protein and/or protein-pigment complexes rather than individual protein molecules. Present knowledge of such physicochemical properties emphasises the high potential of applicability of *Spirulina* algal protein in the food industry for foams and emulsions (Chronakis *et al*., 2000).

Emulsification, water and fat adsorption of Spirulina *algal powder and its protein preparations*

The emulsification properties of proteins are very important to its use in salad dressings, comminuted meat products, cakes and coffee whiteners. The efficiency of emulsification varies with the type of protein, its concentration, solubility, pH, ionic strength, temperature and the method of the preparation of the emulsion. A recent study investigated the functional properties

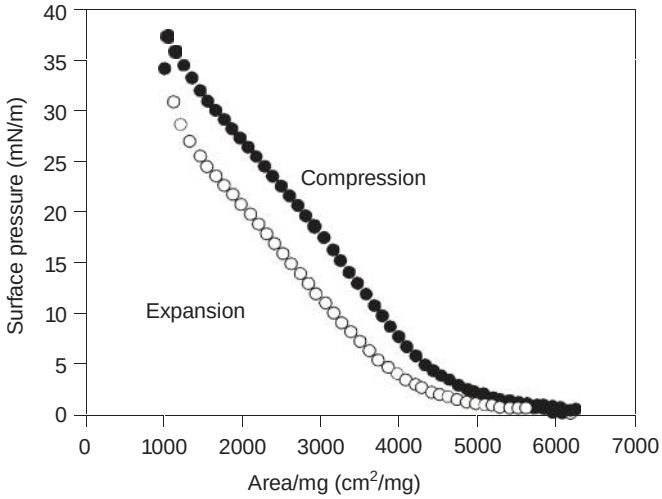


Fig. 14.8 Surface pressure of *Spirulina platensis* strain Pacifica protein isolate as a function of area mg^{-1} for a volume of 100 µ l spread from a solution of 1 mg ml^{-1} of protein isolate dissolved in ethanol: 10 mM sodium phosphate buffer, 50 mM NaCl, pH 7.5 (3 : 1 v/v). Compression (•) and expansion (o). 10 mM sodium phosphate buffer, 50 mM NaCl, pH 7.5 was used as subphase (reprinted from Chronakis *et al*., 2000).

(solubility, foaming properties, emulsification properties and viscosity) of *Spirulina platensis* proteins in relation to changes brought about by chemical treatment with succinic anhydride, acetic anhydride and formaldehyde for succinylation, acetylation and methylation (Mahajan *et al*., 2010).

Protein solubility in an unmodified, water soluble *Spirulina* protein fraction has been found to be 23%. This decreased considerably upon treatment with all the three modifying reagents. The emulsification activity (EA) increased slightly after methylation, while succinylation and acetylation resulted in a decreased EA and emulsion stability (ES) (Fig. 14.9a). The foam capacity (FC) increased after treatment with succinic anhydride at all the concentrations used, whereas acetylation and methylation showed an increase in FC only at lower concentrations (Fig. 14.9b). A maximum FC was found on succinylation and a minimum on acetylation. Foam stability (FS) was found to be much higher with methylation and acetylation. It also appeared that protein molecules of *Spirulina* are unable to dissociate on treatment with the modifying chemicals and hence show greater foam stability. The protein fraction modified with succinic anhydride has demonstrated the maximum viscosity, followed by acetylation of the fraction. Methylation, however, has been observed to cause a rapid decrease in viscosity that was more pronounced at lower concentrations (Mahajan *et al*., 2010).

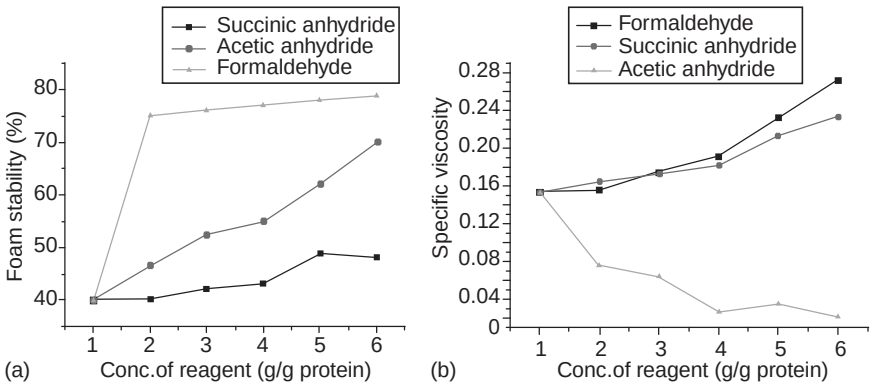


Fig. 14.9 (a) Effect of modification on emulsifying activity of *Spirulina* proteins. (b) Effect of modification on foam capacity of *Spirulina* proteins (reprinted from Mahajan *et al*., 2010).

In another study, water and fat absorption capacity, emulsification capacity, foaming capacity and the stability of flour and protein concentrate of *Spirulina* cells were compared with those of soybean meal (Anusuya and Venkataraman, 1984). The water and fat absorption capacity of *Spirulina* flour were 220 g and 190 g/100 g of the sample, respectively; those of soybean meal were 230 g and 129 g/100 g of the sample. *Spirulina* protein concentrate had a lower water adsorption capacity but a higher fat adsorption capacity than its flour, and the flour had an emulsion and foaming capacity similar to that of soybean meal.

Spirulina powder has a very high foam capacity, especially if the sample is defatted (Nirmala *et al*., 1992). Such a high foam capacity, which is double that of egg protein, appears to be a remarkable property of a spray dried, defatted powder of *Spirulina platensis*. The foam capacity in the same sample without defatting was nearly 50% less. It is not clear whether this is due to a loose lipoprotein complex being formed in the presence of fat, and this may also have some bearing in the drastic reduction of foam properties of proteins. The spray-dried *Spirulina* powder has a much higher emulsification activity and slower kinetics, resulting in higher emulsion stability as compared to a spray-dried defatted sample or to egg protein.

14.5.2 *Chlorella vulgaris*

Evaluations have been made of the capacity of the biomass of the microalga *Chlorella vulgaris* as a fat mimetic and its ability as an emulsifier. Pea protein emulsions with an addition of *C. vulgaris* (green, 60% protein, and orange–carotenogenic, 6% protein) were prepared at different protein and oil contents (Raymundo *et al*., 2005). The addition of *C. vulgaris* proved to

be beneficial in terms of enabling lesser oil contents in the emulsions without disturbing their structural and textural properties. Although the microalgal biomass (Cv green) has a high protein content, it cannot be used as the only emulsifier in these types of emulsion systems. Possible interactions between pea protein and microalgal biomass can also contribute to the reinforcement of the emulsion structure via the formation of physical entanglements. This effect was more significant for Cv green, which must be related to its higher protein content (60% for Cv green vs. 6% for Cv orange). The total oil content can be reduced in this case, yielding emulsions with the same rheological and sensory properties. For this reason, it was considered that the biomass acted as a fat mimetic with a mechanism that likens that of xanthan gum.

The rheological properties of the respective food emulsions were also measured in terms of the viscoelastic properties and steady state flow behaviour and texture properties (Raymundo *et al.*, 2005). The effect of addition of oil on the viscoelastic properties of the 3% pea emulsions with 2% *C. vulgaris* (green and orange) can be seen in Fig. 14.10. These emulsions present mechanical spectra typical of protein-stabilised emulsions in which an elastic network develops owing to the occurrence of an extensive bridging flocculation process. It can be observed from the dynamic measurements that, for a certain protein and microalgae concentration, a higher oil content induces a reinforcement of the emulsion structure. However, texture does not differ between Cv green and Cv orange performance as a fat mimetic; in both cases, the exponential increase of firmness observed with oil contents was not significantly different. Overall, the above results support the potential benefit of using the *Chlorella vulgaris* microalgae to act as a fat mimetic, in addition to the possible advantages as a colouring and antioxidant agent.

14.5.3 Functional properties of other protein microalgal species

The functional properties of the *Porphyridium cruentum*, *Nannochloropsis* spp. and *Phaeodactylum tricornutum* defatted microalgal biomasses have been investigated and compared with those of soybean flour (Guil-Guerrero *et al.*, 2004). On average, *Porphyridium cruentum* dry biomass contains as its major components 32.1% (w/w) available carbohydrates, 34.1% crude protein, 20% ashes and 7% lipids; *Phaeodactylum tricornutum* contains 36.4% crude protein, 26.1% available carbohydrates, 18.0% lipids and 15.9% ashes; *Nannochloropsis* spp. contains 37.6% (w/w) available carbohydrates, 28.8% crude protein and 18.4% total lipids. Guil-Guerrero *et al.* (2004) evaluated the following properties for each microalgal biomass: nitrogen solubility, water and oil absorption capacities, emulsification capacity, viscosity, and sensory evaluation of spaghettis partially containing microalgal biomass. The results showed that *P. cruentum* and *Ph. tricornutum* biomass had functional properties comparable to those of soybean

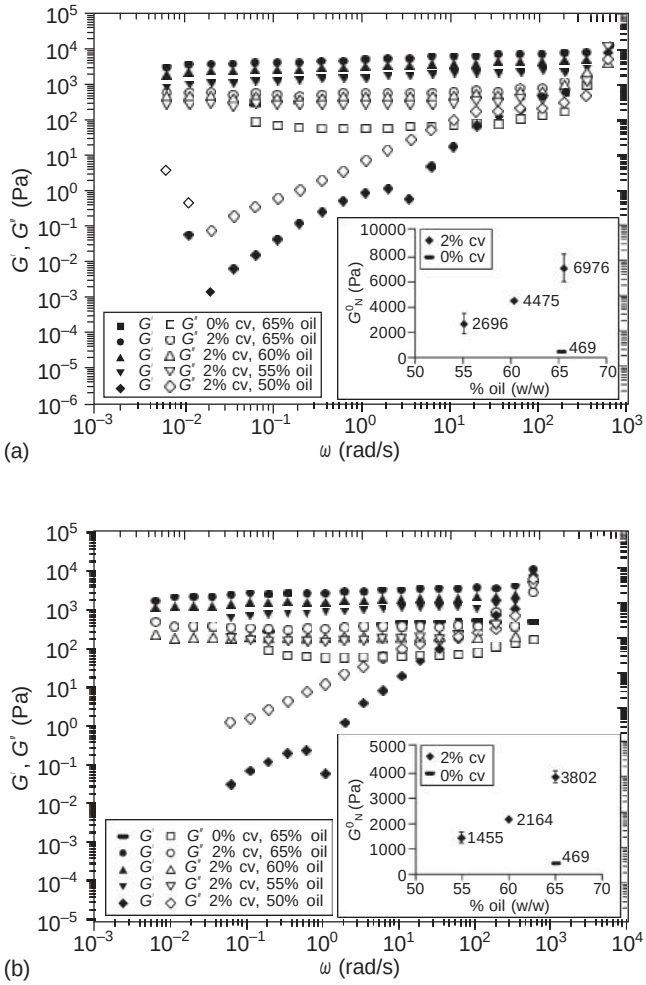


Fig. 14.10 Mechanical spectra of o/w emulsions with 3% pea protein and 2% of *Chlorella vulgaris* orange (a) and *Chlorella vulgaris* green (b), for different oil content and respective values of G^0_N (reprinted from Raymundo *et al*., 2005).

flour. Some functional properties, such as water absorption capacity, showed a different behaviour than that of traditional flours because of the high percentage of exopolysaccharides that the microalgal biomass shows, especially *P. cruentum* biomass. *Nannochloropsis* ssp. biomass requires additional treatment to break its cellular walls before it can be considered possible to use as a functional food ingredient (Guil-Guerrero *et al*., 2004).

In particular, nitrogen solubility is a good index in the design of any potential applications for flour proteins since the percentage of nitrogen insolubility shows a positive correlation with the protein aggregation

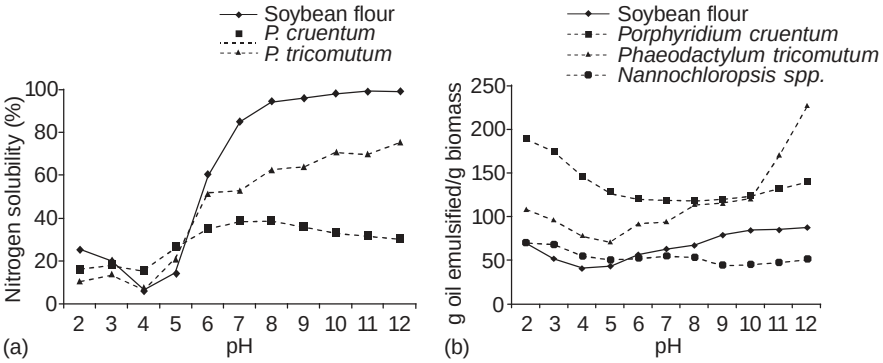


Fig. 14.11 (a) Effect of pH on nitrogen solubility of two microalgal biomasses and soybean flour. (b) Effect of pH on emulsification capacities of three microalgal biomasses and soybean flour (reprinted from Guil-Guerrero *et al*., 2004).

index. Figure 14.11a shows that the minimum nitrogen solubility of the *Ph. tricornutum* and *P. cruentum* defatted biomass were 8.2% and 15.0%, respectively, at pH 4, while that of soybean flour was 8.0 for the same pH. Similar isoelectric points were observed in some legume foods, such as *Psophocarpus etragonolobus*, *Phaseolus calcaratus* and *Dolichos lablab*. A sharp increase in the nitrogen solubility at high pH values was seen for both of the microalgal biomasses used in that study. As suggested, the low solubility of the microalgal protein, especially in the basic pH region, may be an indication that the cell wall inhibits the solubility of the protein. This may occur when the biomass is consumed without there being any process to break the microalgal cell wall and may affect the maximum body utilisation of the protein. This solubility profile agrees with others reported for defatted legume flours with hull. On the other hand, *Nannochloropsis* spp. biomass showed a low nitrogen solubility, which may be due to a particularly strong cell wall that prevented good protein solubilisation at the conditions evaluated. According to these studies, defatted *Ph. tricornutum* biomass can be used in the formulation of acid foods, such as milk analogue products and protein-rich carbonated beverages, considering the finding that the nitrogen solubility was higher than 50% at this pH value for this defatted microalgal biomass, which leads to good biomass solubility (Guil-Guerrero *et al*., 2004).

The plots of the emulsion capacity (EC) vs. the pH of the three microalgal biomasses and soybean flour are shown in Fig. 14.11b. The EC of *P. cruentum* and *Ph. tricornutum* biomasses were significantly higher ($p < 0.05$) than those of *Nannochloropsis* spp. and soybean flour. The minimum EC, at around 50%, was found for *Ph. tricornutum* and soybean flours at pH

4–5. Since the relationships between EC and pH for these were similar to those between nitrogen solubility and pH, EC may depend on the amount of nitrogen that is solubilised. Thus, the minimum EC observed at pH 4 was attributed to the proteins of *Ph. tricornutum* biomass and soybean flour reaching their isoelectric points, which were also at around pH 4–5. As discussed, the high EC observed at the two extreme pH values could possibly be attributed to the higher levels of solubilised proteins, which influenced EC through film encapsulation and a balance of the attractive Van der Waals and repulsive electrostatic forces. On the other hand, *Nannochloropsis* spp. and *P. cruentum* biomasses showed a more similar EC profile, although values of EC were different. The diffuse minimum EC for both microalgal biomasses might indicate that other principles besides the solubilised nitrogen, such as exopolysaccharides, are responsible for the EC of these biomasses (Guil-Guerrero *et al*., 2004)

It was, moreover, observed that the pH and the NaCl concentration significantly influenced the water absorption capacity (WAC) of the three microalgal biomasses. As expected, with the progressive increase in pH, WAC increased to a maximum of 8.1 (*P. cruentum*), 4.5 (*Ph. tricornutum*), 4.4 (soybean flour) and 4.0 (*Nannochloropsis* spp.) (1.0 M NaCl). The high water absorptivity reported suggests that the microalgal biomasses may possibly be used in the formulation of some foods such as sausage, beverages, processed cheese, soups, baked products, etc. (Oshodi *et al*., 1997).

The effect of NaCl concentration on the EC of the microalgal biomasses and soybean flour is shown in Fig. 14.12. As expected, similar solubility

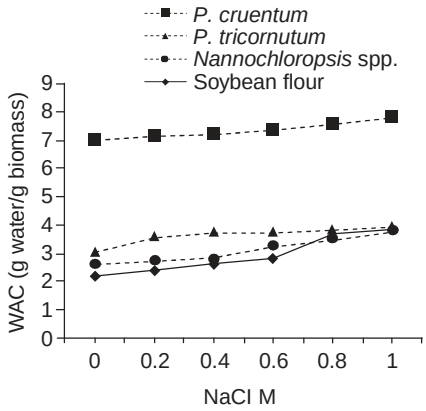


Fig. 14.12 Effect of NaCl molarity on water absorption capacity of three microalgal biomasses and soybean flour (reprinted from Guil-Guerrero *et al*., 2004).

behaviour was found for *Ph. tricornutum* and soybean flour. For these, incorporation of NaCl at concentrations up to 0.4 M had an incremental effect on the EC. EC decreased steadily beyond this salt concentration. In the case of the *Nannochloropsis* spp. and *P. cruentum* biomasses, the addition of NaCl causes only a moderate increase in their EAs, which could be due to exopolysaccharide activity, as previously mentioned.

In addition, sensory evaluation revealed that spaghettis containing different microalgal biomass had characteristics that allow differentiation between them by means of their sensory qualities (Guil-Guerrero *et al.*, 2004). Globally, all spaghettis obtained a good evaluation. The more valued quality of *Ph. tricornutum* biomass was the texture, and it was noted that this microalgae exhibits a pleasant seafood aroma. The colour was especially noted in *Nannochloropsis* spp., while the odour was an excellent sensory property in *P. cruentum*. However, a poor texture was noted in this microalgae, which can be attributed to the previously mentioned high exopolysaccharide concentration. On the other hand, a positive correlation, $p < 0.01$, was found between odour and taste in all cases.

14.6 Nutritional quality of algal proteins

14.6.1 Algal protein digestibility

The nutritional potential of algae as a food protein source differs according to species. A major factor that determines protein quality is the amino acid profile. Another factor equally as important as the amino acid profile is protein digestibility. Even with an excellent amino acid profile, a protein has a low nutritional value if its digestibility is low because of poor bioavailability. The cellulosic cell wall, which represents about 10% of the algal dry matter, poses a serious problem to digestion/utilisation of the algal biomass, because humans and other non-ruminants cannot digest it. Hence, effective treatments are necessary to disrupt the cell wall to make the protein and other constituents accessible to digestive enzymes.

Using polysaccharidases to degrade the algal cell wall has been proposed to improve the availability of the protein in seaweeds (Darcy-Vrillon, 1993; Pohl, 1982). *In vitro* studies that have used enzymatic digestion by proteolytic enzymes such as pepsin or pancreatin and pronase conclude that algae proteins have a high digestibility value (Ryu *et al.*, 1982; Fujiwara-Arasaki, 1979). For instance, *in vitro* digestion tests with pepsin and trypsin have shown that the digestibility of the protein concentrate of *Nostoc muscorum* (a blue-green algae) was relatively high, 74.4% in the cells and 86.8% in the protein concentrates (Yamaguchi, 1996; Mitsuda *et al.*, 1977; Hori *et al.*, 1990). The *in vitro* protein digestibility of *Nostoc commune* was 43–50%.

Fermentation processes have also been used to improve the nutritional quality of algal proteins (Marrion *et al.*, 2003). For example, *Palmaria*

palmata (or dulse), an edible red alga with 8–35% proteins in dry weight, have a weak digestibility because of the cell wall, encapsulating cytoplasmic proteins and limited bioavailability (Bobin-Dubigeon *et al*., 1997). The probable interaction between proteins and xylan also prevents proteolysis during digestion (Galland-Irmouli *et al*., 1999). However, the *in vitro* protein digestibility of fermented samples was 45–65% of that of casein. The improvement observed after fermentation seemed to be due to the degradation of insoluble fibres.

Blue-green algae in general, and *Spirulina* in particular, are unique because they are highly digestible and thus do not require special processing (Yamaguchi, 1996). *Dunaliella* algae are also of particular value in this respect as they lack a cell wall.

Biological indices that are widely used in nutritional studies such as those recommended by the FAO/WHO to evaluate protein quality have also been reported in examinations of the nutritional potential of algal proteins. According to Becker (2007), an accurate method for evaluating the quality of different algae proteins is to determine the protein efficiency ratio (PER), expressed in terms of weight gain per unit of protein consumed by the test animal in short-term feeding trials. However, still more specific nitrogen balance methods can be applied to evaluate the nutritive quality of a protein. One of these principles is estimation of the biological value (BV), which is a measure of nitrogen retained for growth or maintenance. Another parameter that reflects the quality of a protein is the digestibility coefficient (DC). Finally, the net protein utilisation (NPU) – equivalent to the calculation of BV×DC – is a measure of both the digestibility of the protein and the biological value of the amino acids absorbed from the food (Becker, 1986; 1988; 2007; Wood *et al*., 1991). Selected data from such metabolic studies of different processed algae are given in Table 14.6.

Table 14.6 Comparative data on biological value (BV), digestibility coefficient (DC), net protein utilisation (NPU) and protein efficiency ratio (PER) of different processed algae. AD = Air-dried, SD = sun-dried, DD = drum-dried (from Becker, 2007)

Alga	Processing	BV	DC	NPU	PER
Casein	–	87.8	95.1	83.4	2.50
Egg	–	94.7	94.2	89.1	–
Scenedesmus obliquus	DD	75.0	88.0	67.3	1.99
Scenedesmus obliquus	SD	72.1	72.5	52.0	1.14
Scenedesmus obliquus	Cooked-SD	71.9	77.1	55.5	1.20
Chlorella sp.	AD	52.9	59.4	31.4	0.84
Chlorella sp.	DD	76.6	89.0	68.0	2.00
Coelastrum proboscideum	DD	76.0	88.0	68.0	2.10
Spirulina sp.	SD	77.6	83.9	65.0	1.78
Spirulina sp.	DD	68.0	75.5	52.7	2.10

It is evident that the nutritive value of algae proteins is comparable with and in many cases greater than that of most conventional protein feed supplements in term of the gross protein content, unique amino acid quality, composition and biological value, and also in many cases the nutritional acceptability, digestibility and bioavailability of these nutrients, and could make them a potential alternative protein source for human nutrition (Wong *et al*., 2004).

14.6.2 Effect of processing on algal protein digestibility

A number of physical processes (crushing, soaking, drying, grinding, heating, etc.) are widespread in the food industry. These technological processes alter the nutritional quality and digestibility of algae proteins, further demonstrating the important role of proper processing of the algal biomass (Becker, 2007; Marrion *et al*., 2003). The effect of processing on biological value, digestibility coefficient, net protein utilisation, and protein efficiency ratio of different processed algae are also presented in Table 14.6.

14.7 Toxicological and safety aspects

Some safety aspects and toxicological effects that have been widely studied in traditional crops have also been covered in the case of algae and algae protein cultivation, processing and utilisation.

For some algae, such as *Spirulina*, detailed studies have been carried out to characterise the safety of the dry powder form (Chamorro, 1980). An evaluation of commercially produced *Spirulina* found only low levels of mercury and lead contamination that do not indicate a need for restriction as a food supplement at current rates of intake (Hayashi *et al*., 1994). Studies have also evaluated the safety of the *Chlorella* species, including *C. pyrenoidosa*, *C. vulgaris* and *C. regularis*, provided in diets of mice, rats, etc., including pathological investigations, histological examinations, multigenerational growth studies, reproduction studies and haematology studies (Day *et al*., 2009; Cherng and Shih, 2005; Janczyk *et al*., 2006). The results demonstrate that *Chlorella* species provided in the diet are generally well tolerated and do not show any evidence of overt toxicity.

All the animals used in toxicological investigations generally tolerate diets that contain algae very well, even when the algae were present in high concentrations (Becker, 1986; 1988; Chamorro *et al*., 1996; Herrero *et al*., 1993; Noda, 1993). No serious abnormalities have been reported after short-term algae feeding or after a longer period of consumption up to three years. On the other hand, eating large amounts of algae to obtain protein may result in the ingestion of an excessive amount of minerals or ash, leading to diarrhoea. A detailed relevance of algae for health benefits in humans and an adequate safety level remain to be proven and further documented.

Moreover, concentrations of heavy metals in algae production in different locations or caused by environmental pollution may lead to high levels in the algal biomass (Day *et al*., 2009; Ortega-Calvo *et al*., 1993; Karadjova *et al*., 2008). Although heavy metal concentrations in algae have been thoroughly studied in the past two decades (Tripathi *et al*., 2000), a comprehensive description of the mechanism underlining metal toxicity and a gaining of tolerance has not yet been given (Arunakumara and Zhang, 2008). Heavy metals enter algal cells either by means of active transport or by endocytosis through chelating proteins, and they affect various physiological and biochemical processes of the algae.

14.8 Utilisation of algal proteins

14.8.1 Algal proteins for human consumption

The microalgal market is dominated by *Chlorella* and *Spirulina* (Becker, 2004; Pulz and Gross, 2004), not only because of their high protein content and nutritive value but, not least, because they are easy to grow. The biomass of these algae is marketed as tablets, capsules and liquids. Attempts have also been made to include algal material with known food items such as bread, noodles or pasta preparations (Becker, 2007; Hallmann, 2007).

Chlorella health foods are available in the form of tablets, granules and drinks. They came onto the market in Japan in 1964 and sales increased during the 1970s. The first addition of *Chlorella* to foods was in the production of fermented milk by utilising a stimulating effect of a *Chlorella* extract on the growth of *Lactobacilulus* (Mitsuda *et al*., 1961). Nowadays dried biomass and/or extracts of *Chlorella* are used as additives to natto (fermented soybeans) and liquors, because of the effects on micro-organisms, and to drinks, vinegar, green tea, tofu (bean curds), liquors, candies, bread, noodles, etc., owing to the taste and flavour adjusting actions. The rapid spread of *Chlorella* may be due to the fact that various health-promoting effects of *Chlorella* have been clarified.

More recently, microalgae biomass of *Chlorella vulgaris* and *Haematococcus pluvialis* have been studied as a source of natural colourings and fatty acids in a wide range of food products, such as oil-in-water emulsion (Raymundo *et al*., 2005; Gouveia *et al*., 2006), biscuits (Gouveia *et al*., 2007; Gouveia *et al*., 2008a,b) and food gels (Batista *et al*., 2007) with success (Gouveia *et al*., 2008a). As suggested by Gouveia *et al*. (2008c), the addition of *Spirulina* and *Diacronema* microalgal biomass in gelled food systems, such as ''ready-to-eat desserts'', resulted in products with poor sensory properties (particularly colour) in relation to other microalgae (e.g. *Chlorella vulgaris* and *Haematococcus pluvialis*) and other food products (e.g. emulsions and biscuits). Moreover, the gels' colour and pigment content showed good thermal stability when the gelling temperature was increased from 75 to 90°C, revealing an efficient pigment protection inside the *Sp.* and *Di.* cells.

No significant differences ($p < 0.05$) were observed in either algae in terms of the gels' colour and texture properties in the range of concentrations used (0.10–0.75%). It was therefore possible to reduce the amount of microalgal biomass used in order to achieve a desired tonality or texture.

Gouveia *et al*. (2008c) also studied the development of microalgae vegetable-based gelled desserts (similar to ''dairy desserts'') prepared with pea protein isolate, *Spirulina maxima* and *Diacronema vlkianum* biomass, rich in essential fatty acids (omega-3 polyunsaturated fatty acids). The effect of microalgae concentration and gelling temperature on the colour, texture and fatty acid profile of the gels was investigated. When *Di.* and *Sp.* were included in a protein-polysaccharide gelled system, a significant thermal resistance of these biomolecules was observed. A similar effect was previously observed when microalgae were incorporated in biscuit systems. The authors suggest that the resistance of the microalgae bioactive molecules to different heat transfer processes, through ''dry'' and ''wet'' food matrices, is evidence of the potential of microalgae as food ingredients and/or nutraceutical delivery systems.

The commercial algae *Dunaliella* and its water soluble and insoluble fractions were also evaluated for their gross composition and related functional properties when used as a protein supplement in white pan bread (Finney *et al*., 1984). The contribution of the high protein, water insoluble fraction of *Dunaliella* algae to loaf volume was essentially equal to that of the 10% of replaced wheat flour. The chlorophyll of the algae, although probably unobjectionable in very dark breads, would be highly objectionable in light-coloured breads. If algal *Dunaliella* is to be considered as a protein supplement in fermented dough products, it is imperative that the salt be removed (Finney *et al*., 1984).

According to Becker (2007), despite their high content of nutritious protein, dried micro-algae have not yet gained significant importance as food or food substitute. The major obstacles are the powder-like consistency of the dried biomass, its dark green colour and its slightly fishy smell, which limit the incorporation of the algal material into conventional foodstuffs.

Macro-algae are utilised as food in China, Japan, Korea, the Philippines and several other Asian countries. The largest producer is China, which harvests about 5 million wet tonnes/year. For example, "nori", actually *Porphyra* spp., which is used, e.g. for making sushi, currently provides an industry in Asia with a yearly turnover of ~US$1 $\times 10^9$ (Pulz and Gross, 2004). Other species used as human food are *Monostroma* spp., *Ulva* spp., *Laminaria* spp., *Undaria* spp., *Hizikia fusiformis*, *Chondrus crispus*, *Caulerpa* spp., *Alaria esculenta*, *Palmaria palmata*, *Callophyllis variegata*, *Gracilaria* spp. and *Cladosiphon okamuranus* (Hallmann, 2007).

Green algal *Scenedesmus obliuus* was also studied as one potential source of macronutrients in a space habitat. *Scenedesmus obliuus* protein concentrate (70% protein) was incorporated into a variety of food products such as bran muffins, fettuccine (spinach noodle limitation) and chocolate

chip cookies (Nakhost and Karel, 1989). Those food products contained 20 to 40% of incorporated algal proteins. In the sensory analysis, the greenish colour of the bran muffins and cookies was not found to be objectionable. The mild spinachy flavour (algal flavour) was less detectable in chocolate chip cookies than in bran muffins. The colour and taste of the algal noodles were found to be pleasant and compared well with commercially available noodles. Commercially available spray-dried *Spirulina* algal was also incorporated, so the products can be compared with those containing *Scenedesmus obliquus* concentrate. Food products containing commercial *Spirulina* algal had a dark green colour and a 'burnt' after-taste and were less acceptable to the panellists.

Among the green algae, sea lettuce or green laver *Ulva lactuca* is eaten as a salad and in soups (Chapman and Chapman, 1980). Species of *Ulva* are eaten throughout the West Indies and in Barbados. Few among the red algae have been used for food. One of the more important of these in Eire is *Dulse*, or *Palmaria (Rhodymenia) palmata*. Two blue-green terrestrial algae, *Nostoc commune* and *Nematonostoc flageliforme*, are still used by the Chinese of the interior for food. Numerous other eaten species have also been reported (Chapman and Chapman, 1980).

14.8.2 Algal proteins and algae as animal feed

There is now evidence that very small amounts of microalgal biomass, almost exclusively of the genera *Chlorella*, *Scenedesmus* and *Spirulina*, can positively affect the physiology of animals. In particular, a non-specific immune response and a boosting of the immune system of the animals were observed (Belay *et al*., 1993). Such economic effects led to a significant increase in the use of microalgal biomass as feed additives, especially in poultry production. Another very promising application for microalgal biomass or even extracts is the pet food market, where not only the health promoting effects but also effects on the external appearance of the pet (shiny hair, beautiful feathers) are important to consumers. Studies in minks and rabbits provide evidence of such effects in pets (Pulz and Gross, 2004; Kretschmer *et al*., 1995).

Macroalgae such as *Ulva* spp., *Porphyra* spp., *Palmaria palmata*, *Gracilaria* spp. and *Alaria esculenta* are also used as feed for many types of animals: cats, dogs, aquarium fish, ornamental birds, horses, poultry, cows and breeding bulls (Spolaore *et al*., 2006). All of these algae are able to enhance the nutritional content of conventional feed preparations and hence positively affect the physiology of these animals (Hallmann, 2007).

Many other evaluations have shown the suitability of algal biomass as a feed supplement (Becker, 2007). A large number of nutritional and toxicological evaluations demonstrated the suitability of algae biomass as a valuable feed supplement or a substitute for conventional protein sources (soybean meal, fish meal, rice bran, etc.). The target domestic animal is

poultry, mainly because the incorporation of algae into poultry rations offers the most promising prospects for their commercial use in animal feeding. Another growing market is the utilisation of micro-algae in aquaculture. It is estimated that about 30% of the current world algal production is sold for animal feed applications (Becker, 2007).

14.8.3 Utility of phycobiliproteins

Micro-algae represent a potential source of phycobiliprotein pigments, not only for their commercial value but also because these are exclusive to *cyanobacteria* and some *eukaryotic* algae. *Cyanobacteria* contain phycocyanin (blue), allophycocyanin (blue-grey) and phycoerythrin (red) pigments. *Phycobiliproteins* can be used as natural pigments in the food, drug and cosmetic industries to replace the currently used synthetic pigments (Gouveia *et al*., 2006; Cohen, 1986; Moreno *et al*., 1995). *Phycobiliproteins* were used for food colourants in Japan but have not yet been given a GRAS status in the United States.

Spirulina algae contain high levels of the blue biliprotein, phycocyanin (Kageyama *et al*., 1994). A blue food pigmenter manufactured from phycocyanin was marketed. It is a blue powder readily soluble in water that shows an absorption maximum at 618 nm and is stable to light but slightly labile to heat. This product was used as a natural food colour in ice cream, chewing gum, jelly, candy and yoghurt (Kato, 1991). A number of studies have revealed that the oral administration of *Spirulina* exerts diverse therapeutic effects and its addition as a health-promoting agent to noodles and bread has been attempted (Kato, 1992). *Spirulina* also provides an adequate amount of a spectrum of carotenoid pigments, especially β-carotene (associated with cancer prevention) and zeaxanthin (associated with prevention of age-related macular degeneration (AMD)) (Belay, 2002). The green halophilic algal *Dunaliella salina* is as well a good natural source of β-carotene (Borowitzka, 1988; Okuzumi *et al*., 1990) and is grown commercially for use as a dietary supplement and natural food colouring in Australia, the USA and Israel.

A study was also done to determine the effects of *Chlorella vulgaris* biomass as a colouring ingredient in traditional butter cookies (Gouveia *et al*., 2007a). The colour parameters of the cookies remained very stable along the storage period (three months) and a significant increase in their textural characteristics (particularly firmness) was found with an increase of micro-algal biomass added. The biomass incorporation was not detected or was negatively associated with the taste of the biscuits in the sensory evaluation performed.

14.8.4 Recombinant therapeutic proteins from algae

Algae are currently emerging as an alternative system for the production of recombinant therapeutic proteins. Unicellular eukaryotic green algae,

such as *Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum*, *Tetraselmis suecica* and *Odontella aurita* can produce a significant amount of recombinant proteins (Franklin and Mayfield, 2004). The freshwater algae *C. reinhardtii* is the most widely studied for recombinant protein production via chloroplast transformation (León-Bañares *et al*., 2004). *C. reinhardtii* contains a single large chloroplast that occupies approximately 40% of the cell volume, and its transformation was first realised in 1988. Unlike nuclear transformation, plastid transformation occurs via homologous recombination. Hence integration events can be targeted precisely to any region in the chloroplast genome that contains a so-called silent site for transgene integration (Liénard *et al*., 2007).

C. reinhardtii can be grown in a cost-effective manner on a large scale, in 500,000 l containers. Compared to land plants, it grows at a much faster rate, doubling its cell number every eight hours (Franklin and Mayfield, 2004). Purification of recombinant proteins should be simpler in algae than in terrestrial plants. Indeed, the cellular population of algae is uniform in size and type, and there is thus no gradient of recombinant protein distribution, which simplifies purification and reduces the loss of biomass. *C. reinhardtii* also has the ability to produce secreted proteins, a pathway which could further cut production costs (Liénard *et al*., 2007; Mayfield and Franklin, 2005).

Other aquatic plants and green algae (*Chlamydomonas*, *Wolffia*, *Spirodela*, *Chorella*, etc.) can also be used for the production of recombinant proteins (Boehm *et al*., 2001; Kim *et al*., 2002; Franklin and Mayfield, 2005; Sharma and Sharma, 2009).

Overall, there is increasing interest in the use of microalgae for biotechnological applications and as plant model systems. Although biotechnological processes based on transgenic microalgae are still in their infancy, researchers and companies are considering the potential of microalgae as green cell factories to produce value added metabolites and heterologous proteins for pharmaceutical applications. The feasibility of microalgae to be genetically modified and express heterologous genes opens the possibility of enhancing the productivity of traditional algal compounds and producing new bioactive products for industrial and pharmaceutical applications through metabolic engineering (Leon *et al*., 2007).

14.9 Future trends

The full potential of algal proteins as functional ingredients in food products has not yet been realised. It is evident that a number of challenges remain such as the following.

Further development of production processes of algae proteins: although algal proteins can be cultivated and produced in industrial quantities, bulk production of microalgal products still awaits a breakthrough in the design of photobioreactors in which high photosynthetic efficiencies are maintained on large scales and at high light intensities during long term

operation. Production technology must be balanced against cost to achieve the best algal protein system for a particular application. Genetic engineering of algae proteins also holds a promise of new, economically rewarding products and improved yields.

Studies of structural-functional and physiological properties and safety assessments: recent studies of structural-functional properties of algal proteins point to a more complex structural organisation, such as protein-protein aggregates and protein-pigments complexes, and further studies are need to gain information on their structure-functionality relations. Further studies in the functional and health food areas may fill the remaining knowledge gaps in the physicochemical properties, the physiological role that these proteins play in the human diet, the optimum level for algal proteins as a dietary supplement for humans, and their bioavailability. Additional questions must be raised in the safety assessment and the range of values of contaminants, such as heavy metals or minerals potentially present in excess.

The effect of technological processes and post-harvesting treatments on algal proteins: the effect of various physical processes (crushing, soaking, freeze drying, drying, grinding, heating, etc.) that are widespread methods in food industries must be studied further. These technological processes alter the nutritional quality and digestibility of algae proteins and suggest the importance of investigating appropriate processing of algal proteins.

The market for algae proteins will develop steadily, largely due to increased product diversity, and this will also require a change in the use of terrestrial vegetables. Combinations of algae and ordinary plant proteins would improve the nutritional value of foods and at the same time possibly prevent problems related to acceptability and tolerance in the development of food preparations for human consumption that contain algae. It is likely that algal proteins can be a potential dietary and functional food component of immense commercial promise.

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15

Texturized vegetable proteins

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Abstract: This chapter discusses textured soy protein (TSP) also called textured vegetable protein (TVP) and details about the raw materials that can be used to make TSP or TVP. Different steps about processing soybeans into TSP/TVP are discussed in detail. Process details about how to process TVP with extrusion technology are also given. At the end different types of TVP/TSP and their uses are discussed in detail.

Key words: textured soy protein, extrusion, meat analog, high moisture meat analog, fibrous protein, meat extender.

15.1 Introduction

Textured protein products are defined as "fabricated palatable food ingredients processed from an edible protein source including among others soy grits, soy protein isolates, and soy protein concentrates with or without suitable option ingredients added for nutritional or technological purposes." They may appear as fibers, shreds, chunks, bits, granules, slices or other forms. When prepared for consumption by hydration, cooking, retorting or other procedures, they retain their structural integrity and characteristic "chewy texture" (Anon., 1972). The United States Department of Agriculture (USDA) has defined textured vegetable protein products for use in the school lunch program as "food products made from edible protein sources and characterized by having a structural integrity and identifiable structure such that each unit will withstand hydration and cooking, and other procedures used in preparing the food for consumption" (USDA, 1971).

Texturized vegetable protein or "TVP" is a registered trade-mark for texturized soy proteins produced by the Archer Daniel Midland (ADM) company, in Decatur, Illinois, USA. In generic terms, texturized soy protein or "TSP" (a copyrighted trademark of PMS Foods, now Legacy Foods, in Hutchison, Kansas, USA), typically means defatted soy flours or concentrates, mechanically processed by extruders to obtain meat-like chewy

texture when re-hydrated and cooked. A bland-tasting, dried, granular product produced from highly refined defatted soybean meal. It is used in countless meat analog products, from soy burgers to hot dogs, and also as an extender in myriad processed foods from breakfast cereal to frozen desserts.

Texturized vegetable protein is a broad category representing products with a wide array of textures and various processing technologies. For the purpose of our discussion in this text, textured vegetable protein can be described as food items that wholly or partially take the place of meat in the human diet and that have an appearance, texture and nutritional content similar to meat products. Meat products that might be imitated by textured vegetable protein are products made from emulsified meat, such as hot dogs; products made from ground meat, such as sausage or ground beef patties; restructured meat products made from whole-muscle meat such as boneless deli ham; and whole muscle-meat products.

Continuing consumer interest in vegetarianism and more generally in occasional meat-free meals as part of a varied diet are key driving forces behind consumer demand for high-quality and convenient meat alternative products. Industry has responded to this demand with technological developments that enable the use of an increasing range of main ingredients in the manufacturing of meat alternative products (Sadler, 2004). Also, the approval of a health claim for soy-based foods by the US Food and Drug Administration has resulted in increased interest in texturized soy-based products. The term "textured vegetable food proteins" has been loosely applied to a broad range of product categories made from mainly soy flours, concentrates and isolates, as well as other cereal and legume proteins.

15.2 Raw materials for textured vegetable protein

The choice of raw material for texturized vegetable protein depends mainly on availability, cost, functional and physiological properties, nutritional values and custom/tradition. Several sources of vegetable protein can be texturized (Rhee, 2003).

There are four main sources of vegetable proteins:

- Oilseed proteins: Oilseed crops, soybeans, rapeseed/canola, cottonseed, peanut/groundnut, and sunflower seed are the major sources of protein, whereas sesame, safflower, flaxseed and linseed are minor sources.
- Cereal protein: Wheat, corn, rice, barley, oats, sorghum and grain amaranth are the main sources of cereal protein.
- Legume and pulse protein: Beans, gram, guar, lentils, lupines, and peas are the primary sources of vegetable protein in this category.
- Leaf proteins: Alfalfa, lucerne, tobacco, mulberry bush, grass, sugar cane and clovers are the main sources of leaf vegetable protein. However,

currently, there are no leaf protein raw materials available in the market that can be texturized as vegetable protein.

15.3 Soy processing to generate raw materials for texturization

From oilseed sources, soybeans comprise the main crop that is processed into textured protein. This is primarily due to the abundant domestic availability and low cost of soybeans as compared to other oilseed crops. Soybeans are first cleaned, dried, conditioned, cracked and then converted into flakes. Oil is extracted from flakes, and then defatted flakes are ground into soy flour. This is the also the starting material for soy concentrates and isolates. Most textured vegetable protein is made from soy flour and sometime with soy grits. The only difference between flour and grits is the particle size. Grits are classified as coarse (10–20 mesh), medium (20–40 mesh) or fine (40–80 mesh) grits according to particle size (Hettiarachchy and Kalapathy, 1999). Sometimes higher-end textured vegetable proteins, like soy fiber and high moisture meat analogs, are made from soy concentrate and soy isolates. Following is a brief description of each step involved in manufacturing soy flour, concentrates and isolates (Riaz, 2006).

15.3.1 Cleaning of soybeans

The cleaning step is important for producing high-quality end products as well as protecting equipment. Cleaning of soybeans is done on sieves under air aspiration so that dust, plant tissue, pebbles and light contaminating material, as well as bigger impurities (stones, stems, nails, etc.) are separated. The larger impurities are usually separated step-by-step in a de-stoner and a magnetic iron separator. Cleaned beans are weighed with automatic hopper scales, which provide a means to control the rate of feed and the total amount of raw material for accounting purposes. After cleaning the soybeans, the next step is drying them.

15.3.2 Drying of soybeans

Soybeans are dried in grain dryers to a moisture level of 10%, which is needed prior to dehulling. The temperature of the dryer should be between 70 and 76 °C to achieve the desired moisture level. Uniform drying is very important for the removal of soybean hulls, since every individual soybean hull must be removed, not just an average.

To do a good job of hull removal, it is essential to subject the beans to some kind of thermal impact. This is necessary because the hulls are attached to the bean with a proteinaceous material, that when exposed to heat,

affects the release of the hulls. After drying the beans, they are stored for tempering for approximately 72 hours to stabilize the moisture contents. Dried beans are usually cleaned again to remove as many loose hulls, pods, sticks and other foreign material as possible. This step will increase the efficiency of the cracking rolls and aspirator. After drying, the next step is cracking and dehulling the soybeans.

15.3.3 Cracking and dehulling of soybeans

The primary reason for dehulling is to produce high-protein end products. Hulls amount to around 7–8% by volume of the total of bean processed. Removing these hulls prior to processing will increase the protein content of the desired end products. Sometimes, fibers are not required in food applications where these products will be used. Clean dry soybeans are cracked into suitable pieces for dehulling using a cracking mill. There should be a minimum of fines and no mashed beans. After cracking, the hulls are separated under air-flow, and the lighter hulls are withdrawn.

It is important at this point to understand exactly how air separation works, since it is presently the basis for all soybean dehulling. With properly dried and cracked soybeans, the cotyledons should separate easily from the hulls. As a practical matter, it is not possible to get an absolute separation of hulls from the cotyledons.

15.3.4 Conditioning

Cracked and dehulled soybeans are conditioned in cookers to approximately 70 °C with steam. Small amounts of water can be added to adjust the moisture to approximately 11%, which makes the beans ideal for flaking. A correct heat treatment and adjustment of moisture will assist in disrupting the cell membranes during flaking so that the oil can be extracted more easily. The main function of conditioning is to facilitate the flaking step.

15.3.5 Flaking

In order to extract oil from soybeans, it is important to destroy the cell structure of the beans. To achieve this, beans are flaked in the flaking machines, since solvent can flow much more readily through a bed of flakes than through a bed of soy meats or fine particles. The ideal thickness of a flake is between 0.25 and 0.35 mm. Thickness of these flakes depends upon the size of the cracked beans, conditioning and the adjustment of the flaking rolls.

15.3.6 Extraction of flakes

Soy flakes are fed into either a flash desolventizer or vapor desolventizer. In the flash desolventizer, solvent wet flakes go directly from the extractor

into super-heated vapor blown at high velocity through a long tube. Solvent is heated under pressure to 115–138 °C. As this vapor is blown through the tube, flakes are conveyed in and picked up by the vapor stream. In a few seconds, the super-heated solvent will flash off the residual liquid solvent, leaving desolventized flakes to be collected in the cyclone (Fulmer, 1989). Defatted flakes can be milled using a hammer mill to produce soy flour. This flour contains 52–54% protein, as is.

15.3.7 Production of soy protein concentrates

Soy protein concentrates are processed selectively by removing the soluble carbohydrate from soy protein flour by either aqueous alcohol or isoelectric leaching. Soy protein concentrates contain at least 65% protein. Defatted flakes or soy flour are used as starting material. During the processing of soy concentrates, objectives are to immobilize the protein while leaching away the soluble carbohydrates, removing the strong flavor components and the flatulence sugars (stachyose and raffinose). In turn, both protein and dietary fiber contents are increased.

There are several different methods to produce soy concentrates (Lusas and Rhee, 1995):

1. Extraction of flakes with aqueous 20–80% ethyl alcohol.
2. Acid leaching of flakes or flour.
3. Denaturing the protein with moist heat and extraction with water (Ohren, 1981).

Alcohol extraction produces the blandest products. Mild heat drying conditions are used in an acidic water extraction process to retain high protein dispersibility index (PDI) (Lusas and Riaz, 1995a). Isoflavone content in soy concentrate depends on whether it has been water-washed or alcohol-washed, as isoflavones are soluble in alcohol. Because of their blander flavor, soy concentrates are often preferred over soy flour. Typically, soy concentrates are used in meat patties, pizza toppings, bakery products and meat sauces.

15.3.8 Production of soy protein isolates

There are several ways to manufacture isolated soy protein. But the only commercial procedure currently being used is extraction of defatted soy flakes or soy flour with water, followed by centrifugation. In this process, the protein is solubilized at pH 6.8–10 at 27–66 °C by using sodium hydroxide and other alkaline agents approved for food use. The protein solution is then separated from the flakes or flour by centrifugation. The solids are recovered as by-products, containing 16–36% protein, 9–13% crude fiber and 45–75% total dietary fiber when dried to 6–7% moisture content. The solution is then acidified to pH 4.5 by using hydrochloric or phosphoric acid, and the protein is precipitated as a curd. The curd is then washed with water

and concentrated by centrifugation, and can be neutralized to pH 6.5–7.0 or spray dried in its acidic form (Johnson and Kikuchi, 1989). Soy isolates, the most concentrated form of soy protein (90% dry bases), are available.

15.3.9 Mechanical processed soy flour

In this process, soybeans are cleaned, dried, dehulled and cracked (as discussed above), and then fed to a dry extruder. The dry extruder barrel is segmented but not jacketed. All heat for cooking is generated internally by friction, which cooks the soybeans at 325 °F (162 °C) for 25–30 seconds. The extrudate exiting the extruder is in a semi-fluid and frothy state, while the oil is free within the matrix. The soybean extrudate is fed immediately into a screw press, where the oil is pressed out. This technique was first developed by Nelson and his coworkers (1987). The protein-rich meal that comes out of the press in the form of press cake is de-lumped by using a roller mill and passed through a meal cooler. After cooling, this material is ground into flour using a pulverizer. This type of flour will have 6–8% oil depending upon the processing condition. This process of ovoid solvent extraction of the soybeans and resultant material can be classified as chemical free and also suitable for organic soybean processing (Wijeratne, 2000). Researchers at the Food Protein Research & Development Center, at Texas A&M University have modified existing technologies for making extruded full-fat and partially defatted soybean meals, and have added an extruder texturizing step to produce mild-flavored texturized soy products (Lusas and Riaz, 1995b; 1996a; 1996b; Riaz and Lusas, 1997; Riaz, 1999; 2001).

15.4 Processing other crops to generate raw materials for texturization

In addition to soy, some other vegetable proteins have been used for texturization.

15.4.1 Cottonseed

Cottonseed is the world's second major oilseed in tonnage produced and has potential for providing the annual protein needs for approximately 352 million people at the 45 g/day level (Lusas *et al.*, 1989). However, cottonseed contains gossypol, a green-brown compound toxic to humans. Four options for reducing free gossypol content in cottonseed include: binding gossypol by moist heating of seeds flaked to rupture the gossypol "gland" before solvent extraction, extracting gossypol using selective solvent systems, physically removing intact gossypol glands, and growing genetic varieties of "glandless" cottonseed that do not contain gossypol.

Processes have been developed for making glandless cottonseed flour, concentrates, isolates and roasted kernels. Cottonseed accounts for approximately 60% of the weight of seed cotton, but it only provides about 10–15% of the gross returns to producers. Meat extenders have been extruded from cottonseed protein products. Glandless cottonseed flour containing 65% protein may be used to make an acceptable textured product when heavy densities are not required. Insoluble carbohydrates tend to prevent a uniformly layered final product. In general, textured proteins from cottonseed are highly expanded with more cooking loss than similar products made with soy proteins. Availability of glandless cottonseed flour or concentrates is very limited in the market for texturization.

15.4.2 Canola/rapeseed

The problem in rapeseed processing and utilization has been separation of hulls from the small kernels. Textured rapeseed proteins or canola concentrates possess good fat- and water-binding properties. These proteins have excellent nutritional qualities when compared to other vegetable food proteins. Commercially, rapeseed flour or concentrates are very limited and not available for texturization.

15.4.3 Peanuts

Defatted peanut flour has been texturized to produce meat extenders with good taste but poor color qualities compared to soy-based extenders. The Food Protein Research and Development Center at Texas A&M University has done work on texturization of partially defatted peanut flour (Riaz, *et al*., 2005). Partially defatted peanut flour can be produced using the concept mentioned above (for mechanically defatted soy flour). Defatted peanut flour is available in the market, but it is costly, so making a texturized vegetable protein commercially is not economical.

15.4.4 Sesame

The use of defatted sesame flour has received attention due to its nutritional qualities. This vegetable protein, along with sunflower meal, has occasionally been used to produce satisfactory meat extenders. Again, defatted sesame flour is not available commercially for texturization.

15.4.5 Wheat

From cereal sources, the most common ingredient being texturized as vegetable protein is wheat gluten. Other cereals proteins are not utilized currently for texturization. Wheat gluten and wheat starch are economically important co-products produced during the wet processing of wheat flour.

Wheat gluten is a commodity food ingredient, and its applications are predominantly in baked goods and processed meat products. Recent discoveries have found that wheat gluten can be processed into texturized vegetable protein for meat applications. The popularity of textured vegetable proteins that contain wheat proteins is rapidly increasing. Often, these products are extruded in such a way that bundles of long fibers are formed. Wheat gluten can be used in combination with soy-based raw materials, or in combination with wheat flour and other additives to produce a soy-free texturized product. Commercially available wheat gluten is typically 80% protein.

15.4.6 Peas and beans

Pea and bean flour and concentrates have also been used for texturization. These raw materials are somewhat variable, have often been extensively heat treated prior to extrusion and are therefore very difficult to texturize. Pea protein concentrate is available in Canada and Europe and bean flour is available in India.

15.5 Processes for making textured vegetable protein

Raw material for making textured vegetable protein has already been discussed above. A majority of the textured vegetable protein products are produced using extrusion technology. These products are re-hydrated to 60–65% moisture and blended with meats or meat emulsion, which can be extended to a level of 20–30% or higher. For a meat extender, a single screw is used; whereas for meat analogs, protein fibers and high-moisture meat analogs, a twin screw is used. Therefore, it is necessary to begin with an overview of the extrusion principles and methodology of the process.

15.5.1 Principles of extrusion

Extrusion cooking has been defined as "the process in which moistened, expansile, starchy and/or proteinaceous materials are plasticized in a tube by a combination of moisture, pressure, heat and mechanical shear. This results in elevated product temperature within the tube, gelatinization of starchy components, denaturation of proteins, the stretching or restructuring of tractile components, and the exothermic expansion of the extrudate" (Smith, 1975).

Extrusion is widely used to achieve this restructuring of protein-based raw material to process a variety of textured protein. During the extrusion process, mechanical and thermal energy are applied to the proteinaceous raw material, which makes the macro-molecules in the raw material lose their native, organized structure and form a continuous, viscoelastic mass. The extruder barrel, screws and dies align the molecules in the direction of

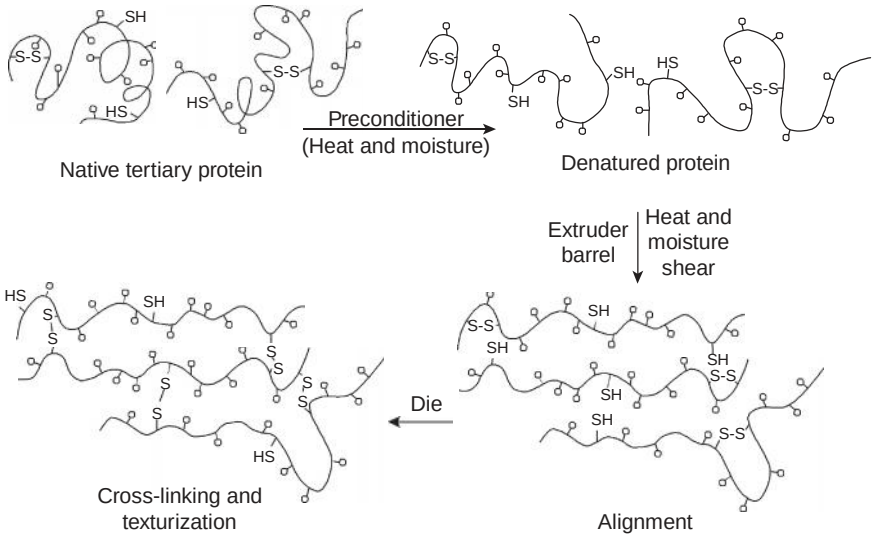


Fig. 15.1 Protein denaturing with extrusion.

flow. This realignment "exposes bonding sites which lead to cross-linking and reformed, expandable structure" that creates the chewy texture in fabricated foods (Harper, 1986). This process is also shown in Fig. 15.1, which explains how native protein, when heated in the presence of moisture and shear, can re-align in the extrusion process.

In addition to retexturing and restructuring vegetable proteins, the extrusion process performs several other important functions (Rokey *et al.*, 1992). Vegetable proteins are effectively denatured during the moist, thermal process of extrusion cooking. Denaturation of protein lowers solubility, increases digestibility and destroys the biological activity of enzymes as well as toxic proteins (Strahm, 2002). At the same time, the extrusion process deactivates residual heat labile growth inhibitors native to many vegetable proteins in a raw or partially processed state. These growth inhibitors have a deleterious physiological effect on man and/or animals as shown by different scientific studies (Rokey *et al.*, 1992).

The extrusion process can control raw or bitter flavors commonly associated with many vegetable food protein sources. Some of these undesirable flavors are volatile in nature and are eliminated through the extrusion and decompression of the protein at the extruder die. The use of preconditioner and an atmospheric venting device in the extruder design may assist in volatilization and removal of off-flavor (Rokey *et al.*, 1992). Extrusion provides a very homogeneous, irreversible, bonded dispersion of all micro ingredients throughout a protein matrix. This not only ensures the uniformity of all ingredients, such as dyes, flavors and other minor ingredients

throughout the product, but provides a means for minor ingredients to intimately associate with potential reaction sites promoting cross-linking or other desirable chemical and physical modifications (Rokey *et al.*, 1992).

15.6 Types of textured vegetable proteins

There are several different types of textured vegetable protein available in the market to be used with meat or "as is". Following is a list of the available textured vegetable proteins (Plattner, 2009):

- High protein snacks
- Chunk-style TVP
- Structured meat analog (SMA)
- Fibrous vegetable proteins
- High-moisture meat analog (HMMA)
- Low-moisture meat analog (LMMA)
- Textured meat protein (TMP).

Each of these textured vegetable proteins is designed for specific application and purposes. The selection of textured vegetable protein depends upon several factors (Riaz, 2009). For example:

What is the application of TSP?
Will this be used in meat system or consumed as it is?
What is the purpose of adding TSP?
What product will it be added to?
What is the requirement of color?
What is the end product utilization?
What are the economics?
What is the final protein content requirement?
What is the final moisture content requirement?
What is the final fat content requirement?
What are the size requirements?
Is flavor is big issue in this product?
What other functional properties are we looking for TSP to provide the meat system?
What are the hydration requirements for those particular products?
Will the fortified product need an approval for child nutrition programs?
What is the standard of identity for the product in which TSP will be used?

15.6.1 High protein snacks

This type of textured protein is designed to be used in dry form for snacks, and can be flavored externally regional flavors. Also, it is used to make high protein snacks like high protein bars. Examples of high protein textured snacks are shown in Fig. 15.2. When texturizing, these high protein snacks

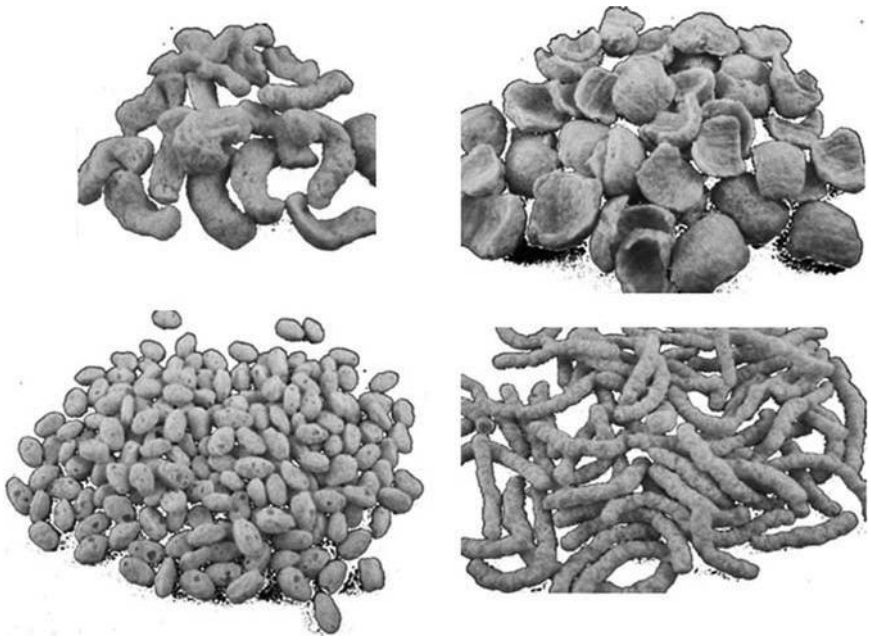


Fig. 15.2 High protein textured snacks.

should be crunchy or crispy but not hard. Since these snacks are consumed in dry form, they should be resistant to breakage during processing. The most common raw material for making these types of high protein snacks is soy flour containing 50–60% protein and 70 PDI. These snacks can be made with soy concentrate containing 70% protein. For this purpose, soy concentrates should be the low solubility type (alcohol washed). Also, the protein content needs to be diluted to avoid over-expansion. As we increase the protein content of these snacks, the texture of these snacks will be affected. Figure 15.2 shows the effect of adding protein in different levels to extrude rice crispies with 80% protein: the texture will change. Sometimes soy isolate rice flour and tapioca starch can be added to achieve the desired functionality. These snacks are externally coated with flavor powders and oil.

15.6.2 Textured vegetable protein (meat extender – chunk style)

Meat extenders produced from the extrusion processing of defatted soy flour or flakes and soy concentrates constitute the largest portion of texturized vegetable food proteins. These types of products are mixed with meat for further processing, changing the properties of the meat. These products are characterized by a random spongy meat-like structure that imitates the

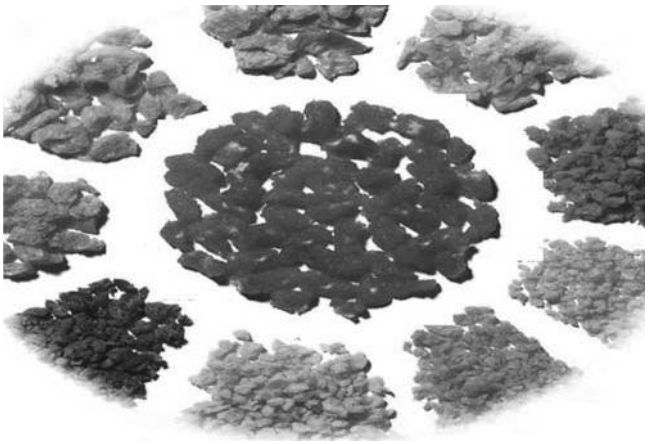


Fig. 15.3 Textured vegetable proteins with different colors.

chewy texture of meat when hydrated with water. These products are re-hydrated to 60–65% moisture and blended with meat or meat emulsions to extend to levels of 20–30% or higher. They are typically purchased in a dry form (6–10% moisture) in sizes that range from small flakes or granules of about 2 mm up to large "steaks" that are 12 mm thick by 80 mm wide by 120 mm long. Other shapes that are typically available are cubes (6–20 mm) and noodles. The products may be colored to mimic a particular type of meat. For example, caramel-colored pieces might be used to mimic a cooked red meat and red pieces might be used to mimic lamb or a cured meat product. Figure 15.3 shows textured vegetable proteins with different colors. As discussed below, these products are hydrated during their transformation into a meat extender or meat alternative. This hydration process typically requires 5–15 minutes in room temperature water and results in the dry product soaking up 1.5–3 times its weight in water. The absorption of water depends upon the raw material used for making textured vegetable protein.

Textured vegetable protein can be made in different sizes ranging from 2 to 30 mm depending upon the application. Meat extenders are available in chunk form (15–20 mm), minced form (>2 mm) and flaked form (>2 mm). Meat extenders can be made from soy flour or soy concentrates, depending upon the nutrition requirements and protein level in the final products. If made with soy flour, meat extenders usually can absorb 2.5–3 times as much water as their original weight; whereas, if made from soy concentrates, they can absorb 4.5–5 times the water. Re-hydration rates depend upon the size and surface area of the products. Extender flakes will re-hydrate more quickly than minced products or chunk style meat extender. The raw material's properties will affect the absorption of water and oil content in meat

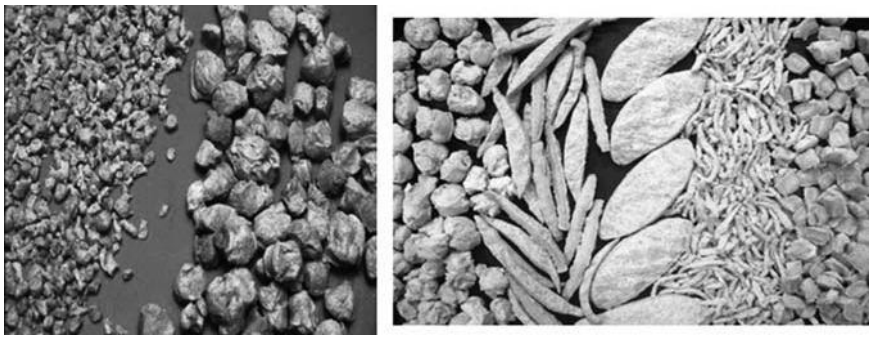


Fig. 15.4 Chunk and minced styles of meat extender and analogs.

systems. Soy flour should have 60–70 PDI and a protein level of 50%. In the case of soy concentrates, a lower protein solubility material can be used in order to make a good product. The resulting product will have approximately 70% protein.

Other raw materials can be added to the recipe for several reasons, including economics, nutritional balance, functionality, color of the final products or to reduce the allergenicity of the final product. Vital wheat gluten, which has high solubility, at least 80% or more protein, can be used with soy flour or soy concentrate to make meat extenders. When making meat extenders, there are several properties to keep in mind, such as water absorption, oil absorption, and meat-like texture. Using proper formulation and processing techniques can help to achieve these properties. Typical chunk and minced styles of meat extenders are shown in Fig. 15.4.

Among meat alternatives, the smaller products in this category are used to manufacture products that imitate meat products made from ground meat. In this case, the granules are hydrated in a mixture of water and flavors and then mixed with binders. These binders are often soluble proteins from sources such as eggs, milk, or soy, but can also include other binders such as starch and carboxymethylcellulose (CMC). This mixture is then formed by pressing into a shape such as a patty or stuffed into a casing like sausage. The formed shapes are cooked to set the binders so that the shape is retained. These meat alternative products are frozen and are commonly available in most US supermarkets as vegetarian meat alternatives. These products can also be used to substitute for ground meat in prepared foods. Examples are a substitute for ground beef in a Southwest dish or canned chili. The larger sized products in this category are often used as basic meat analogs. They are soaked in water and flavors, then packaged or included in a ready-meal product. An example is a 20 mm cube used to imitate chunks of chicken breast in a ready-meal stir-fry product.

These products are typically produced via the extrusion cooking process from solvent extracted soy flour or from soy protein concentrate. Using

these materials, either a single-screw or twin-screw extruder is used. After extrusion, the product may be wet-milled (discussed later) to produce sizes smaller than approximately 8 mm, and then dried to less than 8–10% moisture to make it shelf-stable. Before packaging, the product is sifted into size ranges to remove fines and to produce a uniform size distribution. Products are rarely flavored during the extrusion process due to the flavors being flashed off as the products exit the extruder die. If flavored products are desired, they are manufactured by externally coating a mixture of oil and flavor after drying.

For product developers and consumers who are concerned about genetically modified soybeans, manufacturing can use an identity preserved (IP) program to produce textured soy protein. An alternative method to solvent extraction is available to make soy flour. In this case soybeans are mechanically processed. The mechanically-processed soy flour is made by dehulling the beans, extruding the soy cotyledons to free the oil, mechanically pressing the extruded material to remove a portion of the oil, and then grinding the resulting cake. Texturized products containing up to 10% residual oil have been successfully and uniformly texturized using a properly configured twin-screw extrusion system. Products made from this raw material can be utilized in much the same fashion as those produced from defatted soy flour or from soy concentrate, but they will tend to have darker colors and softer textures due to their extensive heat treatment and lower protein content. A typical composition of textured soy protein is as follows:

- % Moisture, max. 9%
- % Protein (N × 6.25, mfb), min 53
- % Protein (N × 6.25, as is), min 50
- % Fat (pet. ether), max 1
- % Fat (acid hydrolysis), max 3
- % Total dietary fiber 18
- % Carbohydrates (including TDF), by difference 30
- Calories (per 100 gm) 270

15.6.3 Structured meat analog

This type of textured product is remarkably similar to meat in appearance, texture, and mouthfeel when properly cooked. Structured meat analog or (SMA) is a re-introduction of a product that is similar to the Uni-Tex product developed by Wenger in 1975. Raw materials and their properties are very similar to meat analogs and meat extenders. The SMA is characterized by a meat-like, striated layer structure that imitates chunks of whole-muscle meat as shown in Fig. 15.5. This type of product can be made in different sizes ranging from 6 to 20 mm depending upon the application. The density of SMA is much higher than that of chunk-style products already discussed. The only difference between meat analogs and meat extenders is how they are texturized by extruders. If available, it would be



Fig. 15.5 Structured meat analog.

purchased as a dry product of 8–10% moisture. It could be colored to mimic the appearance of cooked red meat or cured meat. SMA usually absorbs at least 3 times the water of their weight, when cooked in boiling water. To transform SMA into a ready-to-eat meat alternative requires hydration in water and flavors, which takes 15–20 minutes under boiling conditions or as much as several hours in cold water. About 1–2 times the dry product's weight in water is absorbed by the dry SMA, which can then be utilized anywhere a diced meat product might be used, such as ready meals or canned products.

A special extruder configuration and die design is used to produce this type of product. When first introduced in the mid 1970s, this product was manufactured using a relatively complex system of two single-screw extruders operating in series. The first extruder served as a pre-cooker and the second as a forming device. Since then it has been discovered that the product can be made using one twin-screw extruder system that relies on good preconditioning with the cooking and forming functions occurring in the extruder proper. The die is designed in a very streamlined fashion to limit expansion that would result in a dense product. The same extruder can be used to make chunk and SMA products. The only differences are the configuration of the extruder barrel, the die designs and processing

conditions. The SMA product is conveyed to a dryer where long retention times are required to remove water from the dense structure. After drying, the product may be sifted to remove any fines, and then packaged. A typical structured meat analog is shown in Fig. 15.5.

15.6.4 Fibrous soy protein

Fibrous protein products are made using a cooking extruder coupled with specialized die technology to form the continuous fibrous structure. These products are characterized by long strands of fine, silky fibers that run lengthwise throughout the piece. A typical recipe for making fibrous protein contains soy concentrate (with high solubility), soy isolates (with high solubility and high viscosity), and starch from corn or wheat. A typical formulation for fibrous protein is as follows:

- Soy isolates 70%
- Wheat starch 30%.

It is a more efficient, high-capacity method for producing products very similar to the spun protein isolates. Fibrous soy protein formation is reliant on the use of specific, high-viscosity soy protein isolates, vital wheat gluten, and starch. Fibrous soy protein products are generally available in chunks that are approximately 15–25 mm in diameter by 30–70 mm long. These chunks are available in a dry (6–10% moisture) form and contain 50–70% protein. A typical fibrous soy protein is shown in Fig. 15.6.

Fibrous soy protein products can also be colored with caramel color to mimic cooked red meat or with red to mimic cured meats. Naturally colored products are very good for producing meat alternatives that mimic poultry or fish products. This type of textured protein is used to restructure the meat analog and mimic the chicken breast type of meat.



Fig. 15.6 Fibrous soy protein.

Fibrous proteins are used to manufacture products that very closely imitate either whole-muscle or restructured meat products such as deli meats, chicken breasts, and fish fillets. To prepare products for consumption, the fibrous chunks are hydrated in water, and usually absorb 3 times their weight in water when re-hydrated. The fibers are extracted from the chunk by a bowl chopper. Desired flavors, color, and binders can be added at this stage. The mass is then reformed into vegetable-based meat analogs such as ham, chicken or beef. These binders are often soluble proteins from sources such as eggs, milk, or soy, but can also include other binders such as starch and carboxymethylcellulose (CMC). The mixture is then shaped into a form such as a simple patty, stuffed into a casing like a sausage, molded into a very complex piece that closely imitates the structural appearance of a half-chicken. The formed shapes are then cooked to set the binders so that the shape is retained. Additional surface treatments may be added to give the appearance of skin or grill marks. These products are very similar to texturized gluten products made by a different process, but entirely comprised of soy material. These meat alternative products are often frozen and can be packaged and marketed in a variety of ways that match the same schemes that are used for the meat products they are mimicking.

15.6.5 High-moisture meat analogs (HMMA)

A relatively new product invented in Europe and then brought to the US, high-moisture meat analogs (HMMA) are presently manufactured by several pilot and commercial extrusion facilities for human consumption as well as for the pet food industry. HMMA is designed to mimic the properties, texture, nutritional profile, flavor, and appearance of whole-muscle meat, especially meat products that are very lean or have low-fat content.

HMMA can be made in various sizes beginning at 12 mm thick by 80 mm wide. It has a densely layered and somewhat fibrous structure similar to that found in whole-muscle products. Typically, it contains at least 60–70% moisture, 2–5% oil, and 10–15% protein. Once it is made, it must be frozen for storage because of high moisture content or retorted in cans for longer shelf life. Generally, it is available in chunks to imitate cubed meat or shreds to imitate pulled meat. Typical applications are prepared ethnic foods, frozen ready-meals and similar high-end consumer food items. A typical HMMA is shown in Fig. 15.7.

HMMA is produced using a cooking extrusion process that relies on a relatively long twin-screw extruder to mix and heat the protein mass at moisture levels of 60–70%. This hot wet mass is pumped by the extruder through a long cooling die where texturization occurs. In addition to texturization, the product is cooled to less than 100 °C before it exits the die so that no expansion occurs, resulting in a very dense product. In addition, since the product is cooled and a large loss of steam (common with the other types of extrusion used for cooked meat alternative ingredients) is



Fig. 15.7 High-moisture meat analog.

avoided, HMMA is one product that can be successfully flavored by mixing the flavor ingredients with the protein material blend.

After exiting the extruder, the product is perishable and needs to be handled just like real meat. It will need to be cooled, shaped, surface treated, and frozen. In addition, retorting and aseptic or chemical preservation are options. A typical recipe contains soy concentrates (high solubility), soy isolate (high solubility/high viscosity), starch (corn or wheat), and oil (from a vegetable source). An example of a HMMA formulation is as follows:

- Soy isolates 45%
- Soy concentrates 45%
- Wheat starch 5%
- Vegetable oil 5%.

15.6.6 Low-moisture meat analog (LMMA)

The low-moisture meat analog (LMMA) is extruded with a twin-screw extruder using a special configuration and die to make a layered/fibrous structure that can mimic whole-muscle meat texture and composition. This



Fig. 15.8 Low-moisture meat analog.

product is cut with an extruder knife at the die into the finished product size and shape, and dried after extrusion for ease of handling, storage, and shelf stability. In terms of size, LMMA can be made from 12 mm thick × 80 mm wide depending upon the application.

This product is hydrated with water to make various meat items. After hydration, its composition is approximately 60–70% moisture, 2–5% oil, and 10–15% protein. Upon hydration, its structure is layered and fibrous, and sizes are very similar to whole-muscle meat. After extrusion, LMMA is dried, cooled, and packed for storage. When needed, it is re-hydrated and then flavored or coated with seasonings and flavors for consumption. A typical formulation of LMMA contains soy concentrates (high solubility), soy isolate (high solubility), wheat gluten, mechanically-expelled soy flour, and oil (from a vegetable source). A typical LMMA is shown in Fig. 15.8.

15.6.7 Textured meat protein (TMP)

This type of product is processed with a twin-screw extruder using soy protein (mainly soy flour) and meat together to mimic whole-muscle meat's texture and composition. The resulting textured meat protein (TMP) is in small random chunks with a very fibrous and layered structure. In this product, low-end fresh meat can be a major part of the ingredients (up to 65% of the recipe). After extrusion, TMP can be dried, frozen, or retort packaged. TMP's sizes and shapes are very similar to "pulled" muscle meat and retort-stable. Its major application is in canned pet food with some other meat applications. A typical TMP is shown in Fig. 15.9.

15.7 Uses of texturized vegetable protein

Texturized soy protein has been a commercial success for many years because of the development of machinery that is capable of continuously producing textured vegetable products. The texturization of plant proteins has been a major development in the food industry. Processes like



Fig. 15.9 Textured meat protein.

extrusion have been developed to impart a fibrous structure to meat. Once texturized, these plant proteins can be dehydrated for use as an extender of fresh or processed meat. Consumers are becoming increasingly interested in healthy foods and open to soy protein ingredients. Texturization of soy flours into usable meat extenders and replacers has been economically feasible for some time. Texturized soy products serve a variety of purposes, including alternative protein sources for the manufacture of convenience foods and for centralized feeding of large numbers of people within defined budgets.

Texturized vegetable protein is being used increasingly in North America as an extender of red meat products. Among the low cost vegetable protein products developed for use in foods as meat extenders or replacement, the most rapid growth has been in the area of textured products manufactured by thermoplastic extrusion. Technology is becoming quite accomplished at creating realistic analogs that match their meat counterparts in terms of flavor, texture and most importantly, satiety. There are analogs of hamburger, both in patties and ground form, sliced lunch meat, sausages, hot dogs, Canadian bacon, pepperoni, bacon bits, and stuffed turkey. Texture

and flavor are the two biggest challenges in developing a meat analog. Vitamins and mineral fortification can be done for the school and military luncheon programs.

Some vegetable protein foods prepared for ordinary consumers are pareve (without meat, milk, eggs and their derivatives) and are of interest to people following Jewish (Kosher) dietary laws. Islam is one of the world's fastest growing religions, and Muslims are today demanding Halal foods. Texturized vegetable foods provide an alternative to animal meat and falls under the Halal foods (Lusas, 1996).

Texturized vegetable protein from soy concentrate has the advantages of blander flavor and major reduction of non-digestible natural sugars (stachyose and raffinose) which can cause considerable flatulence, abdominal discomfort, and venting in some individuals. Modern texturized soy flours have milder flavors than in earlier years, which are easily masked in highly seasoned foods, like tomato sauces, pizza toppings, and canned chilli. Users of texturized vegetable protein include the growing numbers of vegetarians, and people concerned with lowering cholesterol and total fat intake.

Textured soy protein is not a filler; but it can be used as a distinct product, e.g. along with ground beef, as well as simulated products, e.g. a meat analog as a major source of protein in the human diet. Textured soy protein ingredients are nutritious, with some offering specific health benefits, as well as increasing consumer choice; such products therefore have the potential to contribute to overall public health. When textured soy protein is used as a substitute for other products, their textural as well as nutritional properties should be similar to those of the product being replaced. Textured soy protein can be added to meat as extenders or it can be consumed directly as meat analogs. Breaded chicken patties, with as much as 30% of the meat replaced, were actually preferred to all meat patties. Meat analogs can be flavored and formed into sheets, disks, patties, strips and other shapes. There are meat-free hot dogs, hamburgers, chicken patties, nuggets, hams, sausages, meat snacks, and loose meat products for chilis, tacos, and spaghetti. It is very difficult to tell the difference between real and textured soy protein. In India, China, Japan and South Korea textured soy protein is eaten directly as a flavored or seasoned dish usually as a side or main portion of the meal. A good example of a completely meat-free meat analog is flavored bacon bits. Some of the textured protein consumption in different parts of the world is based on religious, cultural, or economic reasons. A good example is vegetarian diets for most (Hindu) Indians. Textured soy protein is widely used in child nutrition programs as well as by worldwide relief agencies to help feed famine-plagued people in impoverished countries. Because of their low moisture and water activity, storage, shelf life and handling under poor conditions do not become a problem. Vitamins and minerals can be fortified in textured soy protein to make it an ideal protein source. It is cholesterol free and can be processed as a low fat food. Textured

soy products can assist in weight control by providing high quality protein in a concentrated form, specially designed low calorie/high nutrient ready-meals can be produced. These can make a significant contribution to weight control products.

Vegetarians have modified many recipes to replace meat with soy proteins. Recipes are available to use textured soy protein in breakfast foods, appetizers, soups, sandwiches, gravies, desserts, ethnic food and main dishes. Several food items are available with textured soy protein, which are designed to grill or cook in microwave ovens. Sometimes these food items are co-extruded with soy concentrates and wheat gluten. Ingredients, including flavors, and colors and edible adhesives (like soy protein isolates, hydrocolloids, or starch, calcium caseinate and egg whites) are added to hydrated textured soy protein before forming into patties, freezing and packaging.

The demand for meat extenders and meat analogs will continue to rise. Meat extenders are still the largest segment of the textured vegetable protein market; however, the use of meat analogs is increasing. We are becoming more aware nutritionally of the foods we eat. Along with the beneficial high protein content of actual meats, there are some negative health benefits, namely cholesterol. However, most people still like their meat. Meat analogs have become a viable alternative in offering a nutritionally acceptable meat substitute that in some cases comes close to matching actual meat products. Food scientists have made major headway in improving flavor, texture, mouthfeel, appearance and color of meat analog products. In the marketplace, you can see more and more meat analog and meat extender products such as bacon bits, soy burgers, meat-free hot dogs, chicken nuggets, breakfast sausage patties/links and bacon to name but a few. Many of these products are even packaged in the same fashion as their meat counterparts. American consumers' acceptance of textured vegetable protein has prompted other countries' interest in this low-cost answer to the desire for more protein foods. The UK, South Africa, Japan, Korea, Mexico and India are among the nations that have joined the United States in commercial production.

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