

Protein Modification During Ingredient Preparation and Food Processing: Approaches to Improve Food Processability and Nutrition

Dongxiao Sun-Waterhouse · Mouming Zhao ·
Geoffrey I. N. Waterhouse

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Abstract Global populations are growing and ageing, with each generation expecting a higher standard of living than their predecessor. Proteins are essential in daily diets because of their nutritional value and roles in food structure, and proteins are generally expensive. Rising awareness of food security and meeting the needs of ageing populations motivate food researchers to explore alternative protein sources and sustainable and optimized use of proteins to increase their bioavailability and digestibility. Proteins behave differently as a function of pH, ionic strength, temperature, pressure or enzymatic mechanism, prompting approaches to modify their structure and functionality. Proteins may lose their native structure and impart sensory changes as a result of processing and storage. Modified proteins are considered as value-added food ingredients, i.e. specialty proteins prepared using enzymatic reactions, hydrolysis, fermentation, heat treatment, acidification, dehydration, emulsification and ultrafiltration. Food formulation influences proteins at both molecular and macropolymer levels. This review presents case studies from previous research and demonstrates approaches for improving protein stability, extractability and bioactivity. The need for combining protein modification and smart food formulation is

highlighted. Synergies achieved between conventional ingredients and added bioactives through careful food formulation and processing are emphasized. Comprehensive characterization of chemical composition, particle size, surface activity, microstructure, freezing-thawing properties of proteins and derived foods is required, to explain structure-function relationships. Data suggest that specialty proteins will play an increasing role in current and future global food sectors. Convenient concept foods for addressing the global food security and ageing population issues are proposed.

Keywords Bioactives · Digestibility · Formulation · Nutritional value · Processing modification · Specialty protein ingredients

Introduction

Growing and ageing global populations present food supply and security challenges. The world population is project to grow to 10 billion people by 2050, requiring at least a 70 % increase in food production (Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat 2009). These challenges, along with the increasing consumer awareness of the relationship between diet and disease/health, stimulate demand for sustainable high quality foods. Proteins are the building blocks of life with fundamental roles in body growth and repair. Thus, protein supply needs to be sustainable, nutritious and environmentally feasible.

Increasing protein supply cannot solely be achieved through increasing animal production, as this would cause issues related to biodiversity loss, fresh water depletion, climate change and human health (Townsend and Howarth 2010). The best solution would be to make five improvements: (1) improve protein expression and production efficiency of proteins in natural plant and animal resources, (2)

D. Sun-Waterhouse (✉) · G. I. N. Waterhouse
School of Chemical Sciences, University of Auckland, Private Bag
92019, Auckland 1142, New Zealand
e-mail: dxsun72@hotmail.com

D. Sun-Waterhouse
e-mail: dx.sun-waterhouse@auckland.ac.nz

D. Sun-Waterhouse · M. Zhao
College of Light Industry and Food Sciences, South China
University of Technology, Guangzhou, China

D. Sun-Waterhouse
The New Zealand Institute for Plant & Food Research Limited,
Auckland, New Zealand
e-mail: Dongxiao.Sun-Waterhouse@plantandfood.co.nz

improve separation and utilization efficiency of proteins from raw materials (including agricultural and industrial waste streams), (3) improve protein ingredient functionality through modification, (4) raise the proportion of plant proteins in human diets and (5) improve the digestibility and bioavailability of proteins in foods during digestion (Payne et al. 1984; Lönnerdal 2002; Vogel 2010; de Jongh and Broersen 2012). The use of breeding and genetic selection and recombinant technology as well as novel protein isolation engineering to achieve high protein content and alter protein structure (the first two improvements) (Richardson 1985; Kim et al. 1989; Weickmann et al. 1994) is feasible but outside the scope of this review. Efficient dietary provision of proteins or amino acids is the first step to ensure high-protein utilization. This requires the development of novel protein ingredients and protein-rich foods, which needs to consider the functional properties of proteins in various real food matrices during food processing (“processability”) and the metabolic pathways of proteins in ingested foods during digestion (“digestibility”), and encompasses the latter three improvements. Other approaches exist to improve food processability and nutrition such as protein-based intelligent packaging, nano-processing and/or nano-formulations that may lead to enhanced functionality and/or reduced toxicity. These novel approaches, although feasible and innovative, are beyond the scope of this review and will not be discussed here.

Discovering new sources of food proteins, besides the conventional plant and animal proteins such as those obtained from meat, fish, milk, egg, wheat and soy, is a key priority for developing new protein ingredients. There exists a global trend towards plant-protein-based diets. There are also many promising opportunities for blending animal and plant proteins in human diets in terms of environmental sustainability, economic profitability, crop-to-human dietary protein conversion and nutritional advantages (Haub et al. 2002; Aiking 2011; de Boer and Aiking 2011). Plant proteins are an economic and versatile substitute for animal proteins as functional ingredients. The consumption of different plant proteins can ensure an adequate supply of essential amino acids needed for human well-being. The food industry can adopt more sustainable practices for the development of novel protein products from both conventional and unconventional origins, e.g. algae, leafs and various seeds.

Proteins are utilized for metabolic flow of amino acids, building blocks of human body, and nitrogenous end products in urine, faeces or sweat. The recommended dietary intake of proteins is about $0.8 \text{ g kg}^{-1} \text{ day}^{-1}$ (Campbell and Leidy 2007). Proteins with different amino acid composition exhibit different digestive behaviour in the gut (Mahe et al. 1996). The extent and pattern of required amino acids are determined by both genotype and factors associated with phenotype such as age, gender, diet, metabolic capacity, body weight and structure, physiological and emotional fitness and lifestyle including workload, physical exercise and resting habits (WHO/

FAO/UNU 2007). Protein quality is measured based on its bioavailability and functionality including digestibility, stability, freedom from interference in metabolism and health benefits. High-quality protein-rich foods are desired by the growing and ageing populations. The elderly people on a high-protein diet may suffer less age-related degenerative loss of skeletal muscle (Campbell and Leidy 2007). Protein-rich foods also have great potential to improve human well-being including their satiety advantage (Bertenshaw et al. 2008; Luhovyy et al. 2007). However, it is challenging to create stable protein-rich foods with desirable sensory and nutritional properties, due to the low stability and tight networks of these foods (Labuza et al. 2007; Labuza 2008).

Nowadays, eating is driven not only by necessity but also by pleasure. Quality, health, taste, convenience and cost drive innovation in food industry. The quality attributes of a nutritious food have now been extended to include inhibition of lipid rancidity, microbial growth and toxin production as well as enhancement of antioxidant activity, bioactive stability and health-promoting functionality. Proteins are essential in foods and play significant roles in food processability, sensory acceptability, nutrition and health functionality (Parada and Aguilera 2007). Proteins have various functional properties including antioxidant, antibacterial, water-holding, foaming or emulsifying capacity (Mendis et al. 2005; Del Rosario Moreira et al. 2011). Native plant proteins and the proteins obtained as by-products from waste streams mostly have limited or undesirable processing and biological properties. The properties of proteins are governed by its amino acid composition and sequence as well as its conformation. Unfolded or hydrolyzed proteins differ in functionality from folded proteins. Thus, the properties of protein ingredients can be tailored through initial optimisation of the isolation and refining processes and subsequent physical, chemical or enzymatic modification. Such modification could improve or create a new type of functionality for different food and nutraceutical applications, through changing amino acid composition or molecule size and/or removing or inserting constituents such as some essential amino acids (Wang et al. 2006a, b; Chobert 2012). Further modification of protein properties could occur, after protein ingredients are added to a food system that is subjected to food processing, storage and digestion. Factors such as protein concentration, surface functional groups, medium/solvent type, pH, salts, hydrophobicity/hydrophilicity scale, pressure, temperature, freeze/thaw cycles and shear can all modify proteins and ultimately the stability and shelf life of protein-containing foods.

This review will describe proteins from the perspective of developing food ingredients and finished foods. This review examines protein structure-function relationships, protein modification approaches, protein digestibility evaluation methods and the synergistic interactions between proteins

and other food components. Owing to the wide scope of this field, this review will focus on exploring the knowledge gap in relation to the demand for new protein-rich foods containing bioactives to improve the wellness of current growing and ageing global populations. The implications of food matrix and food processing on protein nutritional quality and food safety will also be considered.

Protein Sources, Structure and Nutrition

Protein Sources

Protein is globally produced from both animal (~20 %) and plant (80 %) sources for nutrition (Belitz et al. 2009). Amino acids can be sourced from animal products such as milk, meats, fish, eggs, cheese and yoghurt and plant materials such as soy, beans, cereal grains, pulses, nuts, fruits and vegetables (United States Department of Agriculture 2010). Each protein source has unique amino acid composition and thus conveys specific nutritional advantages. While humans require high-quality proteins, there is an upper limit to food protein content (Garlick 2004; Anderson et al. 1993). There exist potential toxicity and human hazards for excessive intake of proteins (this review does not explore this aspect). Table 1 shows that some protein sources provide “complete” proteins and contain all the essential amino acids, while most sources are low or lacking in one or more essential amino acids.

Meat has a relatively high protein content, i.e. 16–40 % depending on species, age and tissue type of animals, providing an important source of amino acids and minerals such as iron, copper, zinc and manganese and vitamins such as B-complex vitamins as well as energy. Meat is not an essential part of the diet but its protein is generally complete and contains “bioactive sequences” that can be released via

proteolysis (Belitz et al. 2009). Different types of meat have different proteins but generally include myofibrillar, sarco-plasmic and connective tissue proteins (Tornberg 2005), which resemble those of human muscles. However, the downside of meat production is the inefficient conversion by animals of feed plant protein into animal protein causing disproportionately large environmental pressures (Stehfest et al. 2009; Steinfeld et al. 2006).

Milk has often been considered as the perfect food due to its excellent nutritional value as well as the true digestibility and good net postprandial protein utilization of its total proteins (~95 and 74 %, respectively) (Bos et al. 1999; Morens et al. 2003). Its composition differs with age and species with a protein range of 3–10 % being typical. A wide range of dairy protein preparations are commercially available including caseins (i.e. in micellar form or co-precipitates), whey protein concentrates, whey protein isolates and specialty whey protein ingredients such as those enriched with β -lactoglobulin, α -lactalbumin and lactoferrin. The functional properties of these milk protein preparations are determined by their protein composition, co-existing non-protein constituents and processing methodologies. Milk caseins and whey proteins are highly valuable protein sources for human direct consumption as well as for producing bioactive peptides to meet the physiologic needs of humans (Choi et al. 2012). Whey proteins are mostly obtained as by-products of cheese making, whereas casein is the solid fraction of skim milk obtained after low pH treatment. Whey proteins accounting for ~20 % of total milk protein are an excellent source of bioactive proteins (Affertsholt and Nielsen 2007). Casein and caseinates are also used for formulating nutritional foods and beverages, although still to a lesser extent than whey proteins. Caseins and whey proteins are considerably different in their amino acid composition and physicochemical properties. Caseins contain phosphorus and consist of four major classes, i.e. α_{s1} -, α_{s2} -, β - and γ -caseins that form micelles and can precipitate at pH 4.6 (isoelectric point of caseins). Whey

Table 1 Limiting amino acids and biological valence of some food proteins (Belitz et al. 2009)

Foods	Limiting amino acid	Biological valence		
		Biological valence (BV)	Net protein utilization (NPU)	Protein efficiency ratio (PER)
Chicken egg		94	93	3.9
Cow's milk	Met (methionine)	84	81	3.1
Fish	Thr (Threonine)	76	80	3.5
Beef	Met	74	67	2.3
Potatoes	Met	73	60	2.6
Soybeans	Met	73	61	2.3
Rice	Lys (lysine), Tyr (Tyrosine)	64	57	2.2
Beans	Met	58	38	1.5
Wheat flour (white)	Lys, Thr	52	57	0.6

Biological valence (BV) = $\{[\text{urea} - \text{N (non-protein diet)} + \text{N balance}] / \text{N intake}\} \times 100$; net protein utilization (NPU) = $[(\text{protein content Gr 2} - \text{protein content Gr 1}) / \text{protein intake}] \times 100$; protein efficiency ratio (PER) = $\text{weight gain (g)} / \text{available protein (g)}$

proteins remain soluble at pH 4.6 and consist of β -lactoglobulin, α -lactalbumin, serum albumin and immunoglobulins as well as various minor proteins like lactoferrin, lactoperoxidase and lysozyme. Differences in amino acid delivery kinetics affect the whole-body protein metabolism of casein or whey proteins by the humans (Boirie et al. 1997).

Eggs are one of nature's near perfect protein foods, consisting of ~13 % proteins and containing other high-quality nutrients (Froning 1994). Egg has been processed into liquid, solid (including powder), frozen products through utilizing whole eggs, white or yolk. These products are commercially used in different food applications such as cakes, pies, noodles, confectionery, salad dressing, powdered soup, desserts and ice creams. Among specific egg proteins, lysozyme separated from egg white is used as a food preservative, e.g. during the later stage of hard cheese fermentation and for meat products to avoid pathogenic bacteria attack (Froning 1994). Egg white is a very useful food ingredient, due to its high bioavailability and essential amino acid contents as well as great food processing functionality including gelling, foaming and emulsifying properties (Powrie and Nakai 1985). New discovery on the positive roles of eggs in the past years would further encourage the inclusion of egg products in healthy human diets, e.g. the findings that a breakfast including eggs results in less craving for a high-calorie lunch compared to a cereal breakfast (Vander Wal et al. 2008) and that egg proteins may improve alertness as eggs whites may stimulate the neurons much more than other nutrients (Karnani et al. 2011). New types of convenient protein food emerge in the market such as the "ips" intelligent protein snacks made from egg whites (<http://www.foodprocessing.com/vendors/products/2013/ips-egg-white-chips/>).

Collagen represents the protein of the greatest quantity in mammals and occurs as the major protein in the connective tissues of the body such as bones, cartilage, ligaments, skin, teeth and tendons. Its modified form via hydrolysis is gelatin. Gelatin contains smaller proteins that are easier to digest and absorb, compared with collagen. The amino acid composition of gelatin depends on the raw material and processing method (Cole 2000). Both gelatin and collagen are inexpensive ingredients for joint health and sports nutrition (Cole 2000; Lugo et al. 2013). Daily supplementation with 40 mg of undenatured type II collagen (UC-II®) was found to improve knee joint extension in healthy subjects and extend the pain-free period of strenuous exertion (Lugo et al. 2013). Further modification of gelatin was also investigated for biomedical applications. For example, modification of gelatin by "click chemistry" under mild conditions, e.g. introducing alkynyl groups through using propiolic acid, could decrease the absolute zeta potential and increase the molecular weight although the charge remains negative and enables its derived hydrogel to possess good reactivity and supports the adhesion and proliferation of chondrocytes (Hua et al. 2011).

Plant proteins are not well utilized yet for human food particularly in western countries, due to their poor nutritional and functional properties as well as low cost-effectiveness of the conventional processing methods (Day 2013). As more advanced processing technologies become available, more plant proteins are entering human diets (Asgar et al. 2010). Vegetable proteins are used to replace meat or soy proteins based on health considerations or eating preferences (e.g. vegetarian) (O'Kane et al. 2004; Moure et al. 2006). Plant proteins are mainly from cereals (57 %) and oilseed meal (16 %) (Belitz et al. 2009). Oil seeds including soybean are one of the most important plant protein sources. Amino acid composition of oilseeds depends on genetic, agronomic and environmental factors such as cultivars, soil type, geographical location, climate and farming methods (Table 2). Soybean has very high annual production worldwide (~271 million metric tons) (USDA-FAS 2012). Soy protein products are gaining increasing importance in food industry. While ~10 % of the soybean crop is used for direct human consumption, 4–5 % of the soybean meal is processed into health-beneficial soy protein ingredients such as soy flour, soy protein isolates or concentrates (Mateos-Aparicio et al. 2008). Soy provides good quality proteins with the highest protein digestibility corrected amino acid score (PDCAAS) among vegetable proteins (Lusas and Rhee 1995). Soybean has 65–80 % storage proteins, i.e. glycinin and β -conglycinin, and their ratio depends on crop year and genotype differences (Khatib et al. 2002). Canola is also globally important containing 17–26 % proteins (USDA-FAS 2012). Canola proteins can be sustainably produced from canola meal by-product of oil extraction especially given the rapidly growing production of canola oil. Sunflower seed has 20–40 % crude protein. Sunflower proteins can be obtained from sunflower meal (a by-product of oil extraction) (Gonzalez-Perez and Vereijken 2007).

Cereals have traditionally been the dominant dietary plant protein source. Wheat is a good example which is consumed by humans in a wide variety of processed foods especially breads, pastas, noodles and cakes (USDA-FAS 2012). Wheat gluten is a group of versatile proteins providing various functional properties in consumer foods, e.g. as fat/water-binders for restructured meat products, or as a meat replacement/analogues in vegetarian foods or seafood (Day 2013). Rice is staple crop in Asian countries despite its relatively low protein content. Presently, a wide range of high-protein rice ingredients (protein > 70 %) are becoming commercially available (<http://www.21food.com>; <http://www.axiomfoods.com/ingredients.php>). These high-protein rice products are produced through extractions at pH 9.5 and/or enzymatic technologies and are considered healthy alternatives (due to low carbohydrate and fat contents) to animal proteins for vegetarians and for consumers who may be allergic to proteins like wheat gluten, eggs,

Table 2 Amino acids in oilseeds (g amino acid/100 g dry mass oilseed)

Amino acid %	Canola seeds	Canola meal	Cottonseed meal	Flaxseed	Sesame meal	Sesame meal (partially defatted)	Soybean meal	Sunflower seeds	Sunflower meal	Dehulled sunflower meal
Arg	1.42	2.12	4.25	1.93	1.82	2.52	3.48	1.84	2.08	2.69
His	0.63	1.01	1.07	0.47	0.65	0.50	1.29	0.58	0.68	0.90
Ile	0.96	1.47	1.29	0.90	2.20	0.73	2.26	0.97	1.15	1.47
Leu	1.61	2.57	2.31	1.24	1.08	1.30	3.70	1.41	1.74	2.26
Lys	1.40	1.89	1.71	0.86	0.71	0.54	2.97	0.79	1.01	1.25
Met	0.47	0.69	0.63	0.37	0.29	0.56	0.65	0.48	0.58	0.76
Phe	0.92	1.43	2.09	0.96	1.18	0.90	2.43	1.04	1.23	1.60
Thr	0.90	1.44	1.21	0.77	1.33	0.70	1.77	0.74	0.92	1.23
Trp	0.31	0.44	0.33	0.30	0.43	0.37	0.65	0.25	0.32	0.43
Val	1.21	1.94	1.79	1.07	2.20	0.95	2.43	1.18	1.43	1.82
Ala	0.99	1.58	1.57	0.93	1.13	0.89	2.06	0.92	1.17	1.50
Asp	1.56	2.45	3.49	2.05	2.93	1.57	5.28	1.88	2.37	3.01
Cys	0.59	0.83	0.65	0.34	0.12	0.34	0.65	0.34	0.44	0.55
Glu	3.81	5.74	7.30	4.04	4.87	3.78	8.35	3.86	4.79	6.23
Gly	1.13	1.79	1.64	1.25	1.30	1.16	2.03	1.17	1.49	1.91
Pro	1.35	2.09	1.36	0.81	0.66	0.77	2.34	0.86	1.08	1.42
Ser	0.77	1.18	1.40	0.97	1.12	0.93	1.97	0.72	0.88	1.18
Tyr	0.64	0.97	1.11	0.49	0.73	0.71	1.73	0.59	0.62	0.85

Data sourced from USDA Nutrient Database; Henkel 2000; Maneemegalai and Prasad 2011

dairy and soy (Shih 2003; Gnanasambandam and Hettiarachchy 1995). *Maize* is an important part of western diets. Commercially available maize protein products include corn flour, corn gluten meal and derived zein protein products (Shukla and Cheryan 2001). *Barley* with 10–17 % protein is also an important cereal crop. Barley's appeal relates to its β -glucan component that has gained a health claim, i.e. “lowering blood cholesterol and glycaemic index” from US FDA. *Sorghum* has a protein content of 9–17 % and plays a crucial role in the global food economy especially in Africa, Asia and the semi-arid climatic regions (International Crops Research Institute for the Semi-Arid Tropics 1996). *Sorghum* is often consumed after fermentation or after cooking (i.e. as porridges or mixing with wheat, maize or rice). *Sorghum* is perceived as an important future food crop for humans, especially as gluten-free products for individuals who suffer coeliac or Crohn's disease and intolerances to wheat (Taylor et al. 2006). Factors affecting its protein digestibility include exogenous factors (grain structure and composition especially polyphenol, phytic acid, polysaccharide components) and endogenous factors (crosslinking via non-disulphide/disulphide bonds, protein hydrophobicity and conformation) (Duodu et al. 2003). Processing also plays an important role, for example, wet cooking, fermentation, sprouting and malt pretreatment could improve considerably the digestibility of sorghum

proteins (Kuo et al. 2013; Raihanatu et al. 2011; Abdelhaleem et al. 2008; Duodu et al. 2003).

Pulses including legumes such as peas, chickpea, lupin, lentil, bean and other dry edible seeds are often served as protein sources, offering various functional and nutritional properties (Gueguen 1991). *Peas* are now being explored for human consumption due to their desirable attributes including their temperate growth climate and healthy nutrients such as protein and fibres (<https://www.goift.com/news/130724-pulse-feature-the-changing-world-of-feed-peas-bard>). Commercial food ingredients such as pea flour, spray-dried or freeze-dried pea protein concentrates and isolates have been developed that have up to 25 % protein (Sandberg 2011). *Chickpea* is a popular pulse crop consumed in countries such as India, Pakistan and Bangladesh, and its protein concentrate products have been commercially manufactured in a similar way to pea protein products (Karaca et al. 2011). *Lupins* have a relatively high protein content, i.e. ~40 % (similar to that of soybean) (Evans et al. 1993), and its white varieties are commonly included in human diet, e.g. in the Mediterranean (Duranti et al. 2008). *Nuts* and *tubers*, especially by-products from nut oil processing and tuber starch production, have been considered as significant protein sources (Camire et al. 2009), although caution has to be taken when utilizing nut proteins in food because of their highly allergic nature (Awad-Allah 2013). Other *unconventional resources* such as cassava root and leaves, agricultural waste and marine plants like algae as

well as *microbial proteins* may become alternative protein sources after appropriate postharvest protein extraction and modification (Ngudi et al. 2003; Nasser et al. 2011). Proteins from unconventional sources must be assessed for safety, nutritional and economic value and environmental impact before mass production is undertaken.

Summary of Protein Composition, Structure and Functional Properties

Proteins, like peptides, are made up of amino acids through amide linkages that are mostly associated with primary amines ($-\text{NH}_2$) and carboxyls ($-\text{COOH}$). Carbon-bonded sulfhydryl ($-\text{C}-\text{SH}$ or $\text{R}-\text{SH}$), thioethers ($-\text{C}-\text{S}-\text{C}-$) and carbonyls ($-\text{CHO}$) functional groups also contribute to protein structural conformation (Belitz et al. 2009). Both the processing and biological properties of proteins are governed by their primary structure (amino acid sequence), secondary structure (α -helices and β -sheets stabilized largely by hydrogen bonds), tertiary structure (3-D organization of secondary structures via disulphide bonds) and quaternary structure (assembly of multiply folded or coiled protein subunits) (Welle 1999). The characteristics of the amino acid side chains such as charge, polarity and size determine the folding of a protein. Met, Glu, Leu and Ala are strongly helix forming while Gly and Pro are strongly helix breaking. Val, Ile and Leu tend to induce pleated-sheet structures, whereas Asp, Glu and Pro behave in an opposite manner. Pro and Gly facilitate turns but Arginine does not (Belitz et al. 2009). Alteration in the secondary, tertiary and quaternary structures, even in the absence of backbone peptide bond cleavage, can cause denaturation (Troncoso and Aguilera 2009). Denaturation of biologically active proteins occurs when the hydrogen, ionic or hydrophobic bonds in proteins are cleaved due to the changes in temperature, pH and interface area and presence of organic solvents, detergents, salts, urea or guanidine hydrochloride, causing loss of activity or improved protein digestion (Damodaran 1996).

Protein solubility in an aqueous solution is critical for protein food processability and thus determines their food applications. Food proteins especially globular proteins gel in water when they are denatured, but are least soluble at their isoelectric point (Li-Chan 2004). Their solubility is affected by ionic strength, pH, food matrix medium and temperature (Li-Chan 2004). Differences in water solubility among plant proteins are of interest. Most plant proteins have extremely low solubility at neutral pH except for soy, pea, lupin and canola proteins (Day 2013). Proteins especially milk and egg proteins are surface active food components due to their amphiphilic nature; thus, they act as emulsifiers to enhance the formation and stability of dispersions such as emulsions and foams (De Jongh and Wierenga 2006). Such emulsifying properties of protein affect food digestibility as well as the

availability of encapsulated bioactives or nutrients (Malaki Nik et al. 2010). Compared to animal proteins, most native plant proteins have very different emulsifying properties, forming thicker interfacial layer because of their bigger molecules and more densely compacted structure due to crosslinking via disulphide bonds (Wong et al. 2012). Good foaming properties are possible for the proteins that are easy-to-denature and have low molecular weight and net charge, high surface hydrophobicity and solubility (Van der Plancken et al. 2007a). Some native plant proteins including pea, lupin, soybean and canola proteins are comparable to egg proteins in foaming (Alamanou and Doxastakis 1997). Hydrolysed or unfolded proteins can induce very different functional properties to food products compared to native folded proteins (Wolynes 2005). Structural modification of plant proteins enhances their surface activities, e.g. deamidated wheat proteins (Day et al. 2009). Protein aggregation generates aggregates with different size and morphology; thus, is important for food and pharmaceutical processing (Zhou et al. 2008; Patil et al. 2011).

Protein Nutritional Value and Digestibility

For humans, new proteins must be synthesized to replace degraded proteins in order to maintain a constant protein mass. The overall protein metabolism in human body is the net result of individual protein metabolism. Individual proteins respond differently to varied stimuli including the combined action of gastric acid and digestive enzymes in the gut lumen and peptidases in the intestinal cells (Welle 1999). Thus, the continuous consumption and turnover of proteins is essential for human physiological maintenance. Five branched-chain amino acids (BCAAs) Leu, Iso, Val, Glu and Arg were found to play critical roles in sports performance (Vary and Lynch 2007). Glu and Arg are essential acting as stimuli for growth hormone release, precursors of antioxidant glutathione that protects cells from free radical damage and modulators for the rate-limiting step of protein synthesis under certain conditions such as stress and exercise (Welbourne 1995; DiPasquale and Mauro 1997; Kanaley 2008). While the amino acid profile determines the overall nutritional quality of dietary proteins especially the concentration of individual indispensable amino acids, protein digestibility and bioavailability reflect the real protein utilization by humans (Boye et al. 2012). The utilization of any protein is first governed by its digestibility.

The human digestive system is directly linked to the vascular, lymphatic and nervous systems by which food intake, digestion and transportation within human body are regulated (Schneeman 2002). Food digestion is a step-by-step process that varies with food quantity, composition, physical state and structure (Weisbrodt 2001). Oral processing of ingested food is the first step where foods are broken down via chewing to

form a bolus mixed with saliva, before swallowing and passing through the oesophagus to the stomach (Lucas et al. 2002). Processing of swallowed foods in the stomach can take from a few minutes to a few hours and is facilitated by the motility of the stomach (i.e. a maximum contraction force up to 1 N) and the secretion of saliva, digestive juices, acids, bile and enzymes (such as gastric lipase and pepsin) (Marciani et al. 2001; Weisbrodt 2001). The dynamics and outcomes of the stomach processing determine the kinetics and pattern of subsequent digestion in the small intestine (Hoebler et al. 2002). Once the chyme reaches the small intestine, digestion of food components continues, with the aid of intrinsic peristaltic motor propelling and digestive juices containing pancreatic enzymes bile salts and mucins, until absorption in the intestinal cells and subsequent transport to the body circulation systems (Salminen et al. 1998). The fate of dietary proteins during digestion is closely associated with the food matrix which they occur. Protein digestion begins in the stomach, but in practice, they are released from food at all stages of ingestion/digestion, depending on solubility and accessibility. Ingested proteins undergo a series of degradative processes catalysed by hydrolytic enzymes such as proteases released from the stomach, pancreas and small intestine resulting in a mixture of amino acids and small di- and tri-peptides that can be absorbed by the small-intestinal enterocytes (Erickson and Sim 1990). Pepsins demand an acidic milieu with the optimal pH in the range of 1.8–3.2 (Untersmayr and Jensen-Jarolim 2008). After the stomach digestion, the remaining proteins and polypeptides in the chyme are further broken down by pancreas-originating proteases and peptidases as well as intestinal mucosa peptidases in the small intestine (Whitcomb and Lowe 2007).

Previous reports suggest that animal proteins have better digestibility than native plant proteins based on conventional food processing technologies, chemical analyses and nutritional evaluation methods (WHO/FAO/UNU 2007; Boye et al. 2012). Whey protein contains more leucine and has a higher total branched-chain amino acid content (i.e. the primary effectors of protein synthesis) than casein and isolated soy proteins (Paul 2009). Isolated soy protein is rich in Glu and Arg and natural bioactives including antioxidants like isoflavones (Paul 2009). Whey protein is considered a “fast” protein because of its fast digestibility and resultant large and transient rise of plasma amino acids, whereas casein is considered a “slow” protein causing a more gradual and prolonged rise of plasma amino acid concentrations (Boirie et al. 1997). Isolated soy protein appears as an “intermediate” protein in between whey and casein proteins in terms of its effect on plasma amino acid contents (Bos et al. 2003; Farnfield et al. 2008). In spite of different digestion rates that affect their ability to promote lean body mass gain upon postexercise protein consumption, milk proteins and isolated soy proteins are thought to be nutritionally complete based on

US Food and Drug Administration labelling guidelines, with a PDCAAS of 1.00 (Dangin et al. 2001). Paul (2009) indicated that isolated soy protein and whey protein show similarity in promoting muscle growth and increasing lean body mass, both outperforming casein proteins. In general, plant proteins have adequate indispensable amino acids to fulfil the normal requirements of adults if the combined use of various plant proteins is adopted (WHO/FAO/UNU 2007). The balanced intake of various plant proteins represents a good approach for complete protein nutrition (Young and Pellett 1994). For special populations, such as infants, growing children and pregnant or maternal women, balanced amino acid composition and easily digestible protein sources are specially required. Blending different proteins such as soy, casein and whey protein offers advantages (i.e. offering more balanced amino acid profile and health benefits), compared to a single type of protein (Henley and Kuster 1994; Paul 2009).

The rate of protein digestion and amino acid absorption determines the delivery extent and rate of amino acid to target tissues and the overall metabolic response, although the impact of physiological differences between human individuals such as age and gender cannot be ignored (Dangin et al. 2001). Small peptides were assumed easier digested because intact di- and tri-peptides were found to absorb after luminal and brush-border peptidase digestion (Grimble 1994), and protein hydrolysates (dominated by di- and tri-peptides) were found to be utilized more effectively than intact proteins and free amino acids in rat trials (Poullain et al. 1989). However, contradictory findings were reported in the literature regarding the difference in absorption of intact proteins and protein hydrolysates. The conflict may have resulted from the different indicative variables employed (e.g. amino acid flux vs amino acid absorption) and rat trials rather than human trials as well as variations in ingestion speed, taste and palatability (Farnfield et al. 2009). For casein proteins, it is likely that hydrolysis facilitates absorption because casein is a slow protein in its intact form (Manninen 2009; Koopman et al. 2009). However, intact whey protein at a concentration of 25 g/500-mL beverage exhibited a more rapid absorption of amino acids into the blood than hydrolysed whey protein at the same concentration (Farnfield et al. 2008). Furthermore, no differences in absorption of amino acids or rate of gastric emptying were detected between whey protein isolate and β -lactoglobulin-enriched whey protein isolate at a concentration of 25 g protein/500-mL drink (Farnfield et al. 2009) and between whey protein or commercially produced whey protein hydrolysate at a concentration of 45 g/500-mL solution (Power et al. 2009). Thus, protein hydrolysates are generally not recommended to be used as meal replacements. It is feasible and probably beneficial to produce hydrolysed proteins, as peptides that are inactive within the protein sequence can be released after hydrolysis and become bioactive, e.g. grass carp protein peptides obtained via hydrolysis with

Alcalase is superior to the intact protein in terms of the physical performance of mice as indicated by swimming prolongation (Ren et al. 2011). More discussions on protein hydrolysates are provided below in the “[Modification of Protein as Isolated Ingredients](#)” section.

While proteins are highly important to human, they also present food allergy risk (Sicherer et al. 2003). Not all the ingested proteins but those resistant to heat, acid or protease digestion/degradation likely cause allergic reactions. These proteins quite often bind to lipids and contain glycosyl groups and multiple disulphide bonds (Lehrer et al. 2002; Yoshino et al. 2004; Van Bilsen et al. 2013). Major food allergens identified include milk, eggs, fish, crustaceans, shellfish, tree nuts, peanuts, wheat and soybean (FDA 2010). Most food allergens are generally glycoproteins ranging from 10 to 70 kDa and containing multiple IgE-binding epitopes (Bannon 2004). Egg allergenic proteins include ovomucoid, ovalbumin, lysozyme and ovotransferrin with ovomucoid being considered the most dominant egg allergen (Mine and Yang 2008). Plant proteins are often inherently stable against normal food processing and digestive conditions, causing allergic reactions and IgE-mediated responses especially for coeliac disease patients (Mills et al. 2004). Some proteins such as storage proteins are more stable to proteolysis in the gastrointestinal tract (e.g. resistant to pepsin digestion) and remain intact or in an immunologically active form, thus sensitizing the mucosal immune system (Moreno 2007). Modification of proteins using crosslinking enzymes transglutaminase and peroxidase can decrease allergenicity of food proteins including soy proteins (Babiker et al. 1998), peanut flour-casein dispersions (Clare et al. 2008), β -casein (Stanic et al. 2010) and wheat proteins (Yong et al. 2006). Food matrix affects the stability of these proteins via interactions with other food components including fats, carbohydrates and proteins (Teuber 2002; Fremont et al. 2004). The presence of lipid phases in digesta can affect dramatically the enzymic degradation of protein allergens, as proteins adsorbed at oil/water interfaces can be partly denatured, decreasing pepsinolysis and promote the delivery of allergens to the small intestine (Moreno et al. 2005a, b).

The concept of “anti-nutritional factors” is also associated with the effect of food matrix on protein digestibility. Anti-nutritional factors are commonly present in high contents in plant products such as tannins, glucosinolates, phytic acid and trypsin inhibitor, which can reduce protein digestibility and amino acid availability (Liener 1980; Gilani et al. 2005). Protease inhibitors (e.g. from legumes) and condensed tannins (e.g. from grape seeds, apple skin and sorghum) would considerably reduce protein and amino acid digestibility through their inhibition of digestive enzymes (including steric effects) and complexation with proteins (Butler et al. 1984). Grinding, cooking or some processing methods could enhance the interaction of tannin with dietary protein thereby decreasing the

protein digestibility (Butler et al. 1984). Flavonoids and phenolic acids containing hydroxyl groups also interact with proteins and form complexes; however, such interactions do not necessarily cause reduced protein digestibility (unlike the tannins). Nevertheless, at neutral to alkaline pH, plant polyphenols can be oxidized to quinones (which can generate peroxides), causing amino acid oxidation, protein polymerization and ultimately decreased protein digestion (Damodaran 1996). Modification of proteins, e.g. introducing bulky and negatively charged side groups via acetylation, methylation or succinylation, limited methylation of proteins or extensive dialysis of proteins after acylation, may facilitate the removal of the anti-nutritional factors through influencing the extent of the protein–tannin, protein–mineral–phytic acid or protein–phenol interactions (Dua et al. 1996; El-Adawy 2000). Novel processing technologies including enzymatic fragmentation and non-thermal processes such as high-pressure processing may reduce protein allergenicity and increase protein digestibility, e.g. through breaking apart epitopes (Lopez-Exposito et al. 2008). More discussions on protein–phytochemical interactions and modification of proteins are provided in latter sections of this review.

Protein digestibility is used to evaluate protein quality and nutritional status of foods. A protein with high digestibility offers a better nutritional value because a greater amount of amino acids is available for absorption on proteolysis (Antunes et al. 1995). Due to the complication of human diet and individual physiological status, accurate measurements of the difference between intake and losses of food proteins to reflect their digestion and absorption have been proven difficult, including studies based on ileal digestibility or faecal digestibility (WHO/FAO/UNU 2007). Biological value (BV) and PDCAAS have been introduced to assess the proportion of absorbed protein from a food to the proteins utilised by the human body and the protein quality based on human amino acid requirements, respectively (WHO/FAO/UNU 2007). BV is greatly affected by the proportion of dispensable and indispensable amino acids and other nitrogen-containing substances (Jackson 1995). PDCAAS value can predict the overall efficiency of protein utilization including digestibility and biological value, i.e. $PDCAAS = \text{digestibility} \times \text{amino acid score}$ (WHO/FAO/UNU 2007). In vitro assays, in vivo animal trials and human clinical trials have been used to examine digestibility of protein ingredients and derived foods (Siu and Thompson 1982). However, care should be taken during data interpretation, as the differences in measured responses or biomarkers and whole system effects (static vs dynamic) can lead to contradictory conclusions. For example, in vivo rat trials showed that the digestibility of almost all the essential amino acids was slightly lowered at high levels of succinylation, but in vitro pepsin and pancreatin digestibility tests indicated the digestibility of Lys, Cys, Met and Thr

amino acids was greatly reduced at high levels of succinylation because the protein was not completely hydrolysed (Siu and Thompson 1982). Also, differences in biologically metabolic systems between human and animals like rats could lead to considerable differences in obtained results, e.g. whey protein feeding on nitrogen balance (Farnfield et al. 2009) and metabolism of succinylated proteins in the absence or presence of a large cecum (Siu and Thompson 1982). In vitro digestion assays that use simulated gastric fluid and single or a mixture of enzymes for measuring the susceptibility of protein to proteolysis still possess advantages for gauging nutritional value, as they are direct, rapid and inexpensive (Haque et al. 1982; Parada and Aguilera 2007). When digestion in the gut is discussed, the oral processing of foods cannot be ignored. As a general rule, where complex real foods are tested, the oral phase is simulated by using a “chew and spit” process (Wickham et al. 2009). Using pepsinolysis to assess protein stability and its relationship to allergenicity is different from the normal in vitro digestion, as it mimics the in vivo environment more closely for investigating and discovering the fate of food proteins and structures in the gut lumen (Wickham et al. 2009). In-depth discussion on protein digestibility is beyond the scope of this review. However, it is important to consider the matrix effect of diet, e.g. co-existing non-digestible carbohydrates or fibres on dietary proteins, which in turn highlights the importance of tailoring food formulations to enhance protein digestibility.

Modification of Protein as Isolated Ingredients

As indicated earlier in this review, there is a need to modify proteins to improve digestibility and nutritional value as well as reduce allergenic and anti-nutritional effects. Unmodified plant proteins normally presents limitations in functional properties or applicability in processed food systems. Moreover, modification of proteins allows efficient isolation and utilization of new proteinaceous materials from unconventional origin and tailors proteins to impart specific and predictable functional properties with less influence by pH, ionic strength, temperature or pressure changes during food processing and digestion. A great deal of work has been done on protein modification but mostly in chemistry or biochemistry laboratory settings with the aims to understand the mechanisms of altering protein structure and function on a molecular basis. Only a few studies were conducted on the nutritional aspects of chemical protein engineering (although based exclusively on in vitro studies) and the effect of protein modification on the physico-chemical properties of proteins. Major approaches for modifying protein as isolated ingredients are grouped as follows.

Chemical Modification

Chemical modification is performed through taking advantage of the chemical reactivity of protein side chains via acetylation, succinylation, lipophilization, glycosylation, thiolation, alkylation, covalent bonding, phosphorylation, amidation or deamidation. Many forces that maintain intact protein structure can be altered, eliminated or created via chemical modification. Chemical modification of proteins, although could alter protein digestibility (Tawfik 2002), presents limitations in large-scale commercial manufacturing due to cost, chemical, consumer and regulatory concerns.

Esterification including acetylation, methylation or succinylation of proteins can block the reactive acid and amino groups of proteins via nucleophilic attack and consequently raised its net positive charge and isoionic points, e.g. esterification with butanol, ethanol and methanol, respectively (Halpin and Richardson 1985). Succinylation can alter the secondary and tertiary structure and/or increase solubility of soy protein (Achouri and Zhang 2001), egg white and whey protein isolate (Zhao et al. 2004), bovine β -lactoglobulin (Stanić-Vučinić and Veličković 2013), flax protein isolate (Wanasundara and Shahidi 1997), wheat gluten protein (Jiang et al. 2005), mung bean protein isolate (El-Adawy 2000), rapeseed preparations (Gueguen et al. 1990; Dua et al. 1996), oat protein isolate (Mirmoghataie et al. 2009) and winged bean protein (Narayana and Rao 1991). In general, succinylated proteins possess improved solubility, emulsifying capacity, heat stability and other functional properties (Sitohy et al. 2001; Wierenga et al. 2005; Mirmoghataie et al. 2009). Acetylation or succinylation leads to elevated protein surface hydrophobicity, which promotes strong network and gelation of proteins (Krause et al. 2001). Esterification may alter protein digestibility in different ways, including increased peptic digestibility of food proteins (Briand et al. 1995), improved digestibility by trypsin, chymotrypsin, and peptidase (Achouri and Zhang 2001), but decreased or unchanged susceptibility to pancreatin, chymotrypsin and pepsin hydrolysis (Matoba and Doi 1979). Highly succinylated proteins may possess good functional properties but could have reduced digestibility (Thompson and Reyes 1980).

Glycosylation is a process of attaching carbohydrates to lysine or the N-terminus. Glycosylation can suppress protein self-association because of the change in kinetic partition between folding and aggregation (Song et al. 2001). Glycation induces major conformational modification causing different shifts of protein isoelectric points and consequently increased or decreased protein stability, e.g. increased thermal stability of apple allergen Mal d3 with which glucose reacts (Sancho et al. 2005), fish proteins (De Jongh et al. 2011) and milk proteins including β -lactoglobulin (Broersen et al. 2004) upon reaction with glucose and fructose as well as decreased β -lactoglobulin after conjugation of glucose (Van Teeffelen et al.

2005). A Maillard reaction intermediate ϵ -N-(furoylmethyl)-L-lysine (furosine) is formed via lactosylation when milk powder is stored (Le et al. 2011). Glycated proteins may display new biological properties, e.g. glycated lysozyme with dextran resulted in a significant anti-microbial activity against Gram-negative and Gram-positive bacteria (Nakamura et al. 1991), and glycated casein with glucose or lactose exhibited enhanced antioxidant activity (McGookin and Augustin 1991).

Deamidation is employed to modify protein solubility, emulsifying and foaming properties (Hamada and Marshall 1989), increase exposed hydrophobicity and surface activity of protein (Matsudomi et al. 1982) or induce protein unfolding (Liao et al. 2010). Both enzymatic and non-enzymatic reactions including acid-induced mechanisms are used for deamidation (Wright and Urry 1991; Suppavorasatit et al. 2011). Most plant proteins are low in water solubility and surface activity at neutral pH due to their high contents of amide-containing amino acids (e.g. Glu and Asp), and acidic or enzymatic deamidation has proven feasible to improve these properties (Suppavorasatit et al. 2011). In some cases deamidated proteins can be obtained directly from food processing e.g. extrusion (Izzo et al. 1993).

PEGylation is accomplished through attaching covalently hydrophilic polyethylene glycol (PEG) to non-specific hydrophobic proteins such as ϵ -amino groups of lysine (Losso and Nakai 2002; Damodaran and Fee 2010). Such PEGylation modifies the water solubility and emulsion stability of proteins (Damodaran and Fee 2010; Losso and Nakai 2002), activity of bovine liver catalase and turnip peroxidase (Abuchowski et al. 1977; Quintanilla-Guerrero et al. 2008), plasma half-life of trichosanthin (He et al. 1999) and stability of lysozyme (Silva Freitas and Abrahão-Neto 2010). Variations of commercially available PEG in terms of oligomer sizes and molecular weights lead to the production of PEGylated proteins with different exposed hydrophobicity.

Thiolation can cause different degrees of modification on the secondary and tertiary structures of proteins, which influences protein aggregation, gelation, self-assembly behaviour and thermal stability that are associated with disulphide interchange (Broersen et al. 2006; Graña-Montes et al. 2012).

Crosslinking through chemical, enzymatic or molecular genetic techniques is an important approach to modify the solubility, foaming, rheological and emulsifying properties of food proteins and improve their nutritional value (by incorporating essential amino acids into low quality proteins like cereal and oilseed proteins) (Li-Chan et al. 1979). Various techniques have been used to introduce new crosslinks into proteins. Most protein-crosslinking reagents react mainly with amino or sulfhydryl groups, although some react with carboxyl or guanidino groups (Han et al. 1984). Esters of imidic acids and of *N*-hydroxysuccinimide are the most commonly used crosslinking reagents, due to mild reaction conditions and

commercial availability. Different aldehydes such as glutaraldehyde and formaldehyde are also used to induce crosslinks (Migneault et al. 2004). Attention should be paid to the use of aldehyde crosslinking agents for food applications due to the potential toxicity. Transglutaminase-catalysed protein crosslinking does not harm human beings (Seguro et al. 1996b; Hultsch et al. 2005), and thus, it is suitable for food applications. Transglutaminase-catalysed crosslinking protein products, although could be digested slower, have similar nutritional value and bioavailability to untreated proteins (Tang et al. 2006; Seguro et al. 1996a).

Lipophilization refers to covalent linkage of lipids (e.g. saturated or unsaturated fatty acids) to proteins, which alter surface properties, e.g. increased protein exposed hydrophobicity (Liu et al. 2000; Wilde et al. 2004; Aewsiri et al. 2011). Lipophilization of soybean glycinin with palmitic acid progressively improved foaming stability and emulsifying activity (Haque et al. 1982). The lipids and proteins in foams may oxidize at the air-water interface causing reduced surface activity due to the presence of hydrophilic antioxidant (Aewsiri et al. 2013).

Hydrolysis

Bioactive peptides are commonly inactive within a protein sequence but exhibit biological efficacy after being liberated from their protein precursors (Korhonen and Pihlanto-Leppälä 2001). Proteins can be hydrolysed using acid, alkali or enzymes to produce hydrolysates containing short-chain peptides and amino acids which are used as an alternative to intact proteins to formulate foods. These hydrolysates or their peptide components possess desirable nutritional attributes and bioactivity including ease of impaired luminal hydrolysis and absorptive capacity, and antihypertensive, immunomodulatory, antioxidant, anti-cancer, anti-thrombotic, anti-fatigue and anti-microbial activities (Frokjaer 1994; Van Beresteijn et al. 1994; Clemente 2000; Elias et al. 2008; You et al. 2010a, b; Piotta et al. 2012). A large number of published studies describe the effect of proteolytic hydrolysis of food proteins on their functional properties, including animal proteins such as casein (Chobert et al. 1987; Smyth and FitzGerald 1998), and plant proteins such as soybean proteins (Mohri and Matsushita 1984) and wheat gluten (Wang et al. 2007a, b). Zein, sunflower globulins and milk proteins (casein and whey) are starting materials suitable for producing protein hydrolysates with high branched-chain amino acid contents that are highly desired by patients with hepatic failure (Tanimoto et al. 1991; Bautista et al. 2000; Clemente 2000; Farnfield et al. 2009). All protein hydrolysates are not created equal even though they are from the same sources and have the same degree of hydrolysis (but made by different methods) (Grimble 2000). Enzymatic hydrolysis of proteins quite often causes decreased molecular weight and increased ionization

and exposure of buried hydrophobic groups (Panyam and Kilara 1996). New or enhanced food processing and biological properties were observed after such hydrolyses (Table 3). Peptides derived from proteolysis may impart different tastes to foods such as bitter tastes in cheeses due to faulty ripening or sweet tastes due to L-aspartic acid; thus, bitterness masking may be needed after the bitter peptides are released during food development (Belitz et al. 2009; Nielsen 1997; Pedersen 1994).

Protein hydrolysates produced by *acid and alkali hydrolysis* often have limited commercial value due to their decreased nutritional qualities, for example, damage of L-amino acid and generation of D-amino acids and toxic lysinoalanine (Lahl and Grindstaff 1989). In comparison, *enzymatic hydrolysates* from various protein sources have great marketing potential, due to high product quality (as a result of enzyme catalytic specificity), high production speed (10^8 – 10^{11} times faster than the corresponding non-enzymatic reactions), moderate production cost and conditions (pH 6–8 and 40–60 °C), commercial availability and regulatory authorization of enzymes (Nielsen 1997; Clemente et al. 1999a, b). Food enzymes under different legislation systems are listed as food additives (Regulation (EC) No 1333/2008) or processing aids (Regulation (EC) No 1332/2008). Enzymes are proteins that adopt a unique 3-D conformation connected via non-covalent interactions such as hydrogen bonds to enable their catalytic activity and specificity. Environmental pH or temperature influences the enzyme's conformation and site for binding substrates, causing stability reduction or complete loss of enzyme activity (Sun-Waterhouse 2013). The resultant protein hydrolysates contain desired amino acid composition and molecular size, exhibiting improved absorption efficiency, solubility, heat stability, emulsifying or foaming property and resistance to precipitation (Nielsen 1997; Popineau et al. 2002; Ribotta et al. 2012). The determination of the type of enzyme and the hydrolysis conditions is based on the end use of protein hydrolysates, e.g. food applications. For example, trypsin or Alcalase may hydrolyse soy protein isolate more effective than chymotrypsin or rennet; furthermore, the solubility of the resultant hydrolysates by trypsin and Alcalase even though are similar at pH 4.5 can be totally different at pH 7.0 (Kim et al. 1990). Peptide fractions isolated from the same protein hydrolysate may also exhibit different levels of functionality, e.g. shorter peptide chain had higher reactivity for Maillard reaction (Fig. 1, Su et al. 2011). A sequential modification approach, i.e. initial use of endopeptidases followed by exoproteases (Clemente 2000), followed by posthydrolysis treatments such as heat inactivation, separation, debittering or bitterness masking, is often employed to achieve a more complete hydrolysed product with desired molecule size and sensory attributes (Pedersen 1994). It is worth noting that bioactive peptides may also be generated during *in vivo* gastrointestinal processes by digestive enzymes like trypsin and

pepsin (FitzGerald et al. 2004; Korhonen and Pihlanto-Leppälä 2006) and during food processing (Yamamoto et al. 1994; FitzGerald and Murray 2006).

Intact milk proteins consist of various bioactive peptides; thus, production of bioactive peptides by using casein and whey proteins as precursors present huge opportunities (Meisel and Schlimme 1990). For example, a whey protein hydrolysate Lacprodan® 3065 from Arla Foods Ingredients has recently gained China's approval for listing on food product labels. This presents food manufacturers with massive opportunities for using whey protein hydrolysates in foods for easy digestion with low allergy risk (<http://www.arlafoodsingredients.com/news/talking-point/whey-protein-hydrolysate-gets-green-light-in-china>). Casein is heat-stable with low solubility at its isoelectric point (pH 4.6) (Belitz et al. 2009). This restricts the application of casein to high acid foods and beverages. Limited enzymatic proteolysis of casein could reduce molecular weight to <15,000, increase solubility at pHs of 4.0–5.0 and improve gelling and emulsifying properties (Chobert et al. 1988a, b; Nakai and Li-Chan 1989). Whey has been processed into different food ingredients such as whey protein concentrates or isolates by simple drying and separation of non-protein compounds like lipids, minerals and lactose (Timmer and van der Horst 1997) or whey hydrolysates by enzymatic hydrolysis using pepsin, trypsin, chymotrypsin, and other plant, bacterial or fungal proteases to confer new or improved nutritional and processing properties (Foegeding et al. 2002; Nakai and Li-Chan 1989). The bitterness of whey protein hydrolysates present sensory issues for their derived food products, which can be reduced via controlling the degree of hydrolysis and using ultrafiltration (Ziajka and Dzwolak 1999).

Heating

Proteins are sensitive to changing temperature (Belitz et al. 2009); thus, it is feasible to use heating to modify proteins. Controlled heating can modify proteins to achieve special functional properties and digestibility (Genovese and Lajolo 1996; Duodu et al. 2003). For example, a two-step controlled heating method can produce egg white gel with different degrees of transparency over a wide range of NaCl concentrations (Murata et al. 1993; Tani et al. 1993). Heat-induced Maillard reactions are also possible, which may increase or decrease the nutritional properties of protein food products (Friedman 1996; Liu et al. 2012).

In summary, modification of proteins offers an effective approach to utilize readily abundant and relatively cheap protein resources to produce specialty protein ingredients with excellent nutritional value, digestibility and processing properties (De Jongh and Broersen 2012). The combined use of heat treatment and limited proteolysis followed by ultrafiltration can improve protein emulsifying properties via

Table 3 Different protein enzymatic hydrolysates and their properties

Source	Protein hydrolysate	Functionality	References
Milk	Whey hydrolysate	Improved flavour, texture and stability of snack bars	Adams. 2008.
Milk	Hydrolysate of whey protein isolate	Lowered water activity	McMahon et al. 2009.
Milk	Whey protein hydrolysates	Preventing water migration to protein during snack bar storage	Gautam et al. 2006.
Milk	Hydrolysate of milk protein by Alcalase and Neutrase	Decreased water holding capacity, but remarkably increased protein solubility	Mietsch et al. 1989.
Milk	Hydrolysate of whey protein concentrate by papain and a fungal protease	Increased water-holding capacity and protein solubility, preventing moisture migration in snack bar and slowing bar hardening.	Sinha et al. 2007.
Milk	Hydrolysate of milk protein concentrate by trypsin or chymotrypsin	Increased digestibility to nurture a young calf	Guo et al. 1995.
Milk	β -lactoglobulin by <i>Lactobacillus paracasei</i> peptidases	Induce in vivo oral tolerance to β -LG and releasing immunomodulatory peptides that stimulate regulatory T cells (major immunosuppressive agents by secreting IL-10).	Prioult et al. 2004.
Milk	β -lactoglobulin and alpha-lactalbumin enzymatic hydrolysates e.g. by tryptic digestion or chymotryptic hydrolysis	Anti-bacterial (against Gram-positive bacteria)	Pellegrini et al. 1999, 2001.
Milk	Hydrolysate of glycomacropeptide in whey protein concentrates or isolates (by endoproteinase Glu-C)	Growth-inhibitory activity against <i>Streptococcus mutans</i> .	Malkoski et al. 2001.
Milk	Hydrolysed whey protein isolate	Increased plasma leucine, branched-chain amino acids and total amino acids	Farnfield et al. 2009.
Milk	Casein hydrolysed by transglutaminase	Decreased allergenicity	Yamauchi et al. 1991.
Milk	Hydrolysate of milk, e.g. tripeptides, Ile-Pro-Pro and Val-Pro-Pro	Anti-hypotensive: reduce systolic BP by -28.3 and -32.1 mmHg, respectively, in spontaneously hypertensive rats	Nakamura et al. 1995.
Milk	Casein hydrolysates by different proteases	Antioxidant and anti-inflammatory activities in human Jurkat T cells by increasing cellular catalase activity and amount of reduced glutathione	Phelan et al. 2009; Lahart et al. 2011.
Milk	Casein hydrolysed by transglutaminase	Decreased allergenicity	Yamauchi et al. 1991
Milk	Caseins hydrolyzed with <i>Lactobacillus</i> GG and digestive enzymes	Suppressive effects on lymphocyte proliferation	Sutas et al. 1996.
Milk	Enzymatic hydrolysates of <i>Lactobacillus</i> GG fermented UHT-milk	Immunostimulating properties	Rokka et al. 1997.
Milk	Lactoferricin hydrolysates	Anti-inflammatory and immunomodulating properties or for chemoprevention of carcinogenesis	Vogel et al. 2002.
Milk	Tryptic hydrolysates of κ -casein	Anti-thrombotic activity by inhibiting fibrinogen binding platelet	Jollès and Henschen 1982; Jollès et al. 1986.
Milk	Pepsin hydrolysate of bovine lactoferrin	Anti-microbial potency	Tomita et al. 1994; Dionysius and Milne 1997.
Milk	Caseins hydrolysates by digestive enzymes or fermentation of milk with proteolytic lactic acid bacteria strains	Improved anti-atherogenicity and Antioxidative activity e.g. free radical scavenging activities and to inhibit enzymatic and non-enzymatic lipid peroxidation	Korhonen and Pihlanto-Leppala 2003; Kullisaar et al. 2006; Rival et al. 2001.
Milk	Whey fermented by <i>Lactobacillus helveticus</i> CPN4	Antihypertensive effect	Yamamoto et al. 1999.
Milk	Casein or whey protein hydrolysate	Lowered blood pressure in humans	FitzGerald et al. 2004.
Milk	Milk protein hydrolysate	Anti-hypertensive, antioxidant, immunomodulatory, anticancer, antimicrobial, and lipid-lowering activities	Meisel 2004; Pihlanto 2006
Milk	Limited hydrolysis of casein with <i>Staphylococcus aureus</i> V8 protease	Increased the isoelectric solubility	Chobert et al. 1988a.
Milk	Limited hydrolysis of casein with enzymes such as trypsin or Protamex	Improved emulsion stability or emulsifying activity	Chobert et al. 1987, 1988b; Slattery and FitzGerald 1998.
Milk	Protamex hydrolysates of sodium caseinate	Increased foam expansion, or improved foam formation and stabilization	Slattery and FitzGerald 1998; Caessens et al. 1999.
Milk	Hydrolysate of whey protein concentrate with a glutamic acid specific protease from <i>Bacillus licheniformis</i>	Increase of viscosity and production of plastein aggregates	Budtz and Nielsen 1992.

Table 3 (continued)

Source	Protein hydrolysate	Functionality	References
Milk	Whey protein hydrolysate or fermentation of whey proteins	Immunoreactivity reduction of native allergenic proteins, or removal of antigenicity	Bu et al. 2010; Jedrychowski and Wróblewska 1999.
Milk	Casein hydrolysate by chymosin, chymotrypsin or Pepsin	Anti-fungal activity	Lahov and Regelson 1996.
Milk	Hydrolysate of heat-denatured whey proteins	Improved interfacial properties e.g. surface hydrophobicity	Mutilangi et al. 1995.
Milk	Hydrolysate of caseins or whey proteins by “Corolase PN”, bromelain, trypsin or a <i>Bacillus subtilis</i> protease	Improved water-holding capacity	Kneifel and Seiler 1993; Ju et al. 1995.
Milk	Limited proteolysis of whey protein	Improved foaming properties	Kuehler and Stine 1974.
Meat	Beef sarcoplasmic protein hydrolysate	Anticancer, antimicrobial and ACE-inhibitory properties	Jang et al. 2008.
Meat	Hydrolysate of chicken breast meat proteins by Alcalase	Increased soluble nitrogen and formaldehyde nitrogen	Cui et al. 2009.
Meat	Loach protein hydrolysates	Increased hydroxyl, ABTS radical scavenging activity, reducing power and chelating ability of Cu ²⁺	You et al. 2010a.
Meat	Loach protein hydrolysates by papain	High in vitro DPPH, hydroxyl radical antioxidant activity and chelating ability of cupric ion, and in vivo anti-fatigue activity	You et al. 2011.
Egg	Hydrolysate of egg albumin gels	Improved gel characteristics	Kitabatake and Doi 1993.
Egg	Hydrolysed hen egg white lysozyme	Multifunctionality as CaMPDE-inhibiting antioxidants	You et al. 2010b.
Egg	Hen egg hydrolysate	Possess immunostimulating activities and increased immune functions during cancer immunotherapy	Mine and Kovacs-Nolan 2006.
Marine	Chum salmon hydrolysed by complex protease	Immune stimulant: enhanced mitogen-induced lymphocyte proliferation, natural killer (NK) cell activity; multifunctional property, e.g. Protected against gamma radiation induced immunosuppression and antioxidant	Yang et al. 2009, 2010; Liang et al. 2010.
Marine	Pacific oysters hydrolysed by crude protease solution from <i>Bacillus</i> sp. SM98011	Dose-dependent inhibition of growth of sarcoma in mice; enhanced NK cells activity, spleen lymphocyte proliferation, and macrophage phagocytic rate	Wang et al. 2010.
Marine	Jellyfish collagen hydrolysed by Protamex	Decreased serum and hepatic malondialdehyde; increased glutathione peroxidase and superoxide dismutase	Ding et al. 2011.
Marine	Seahorse hydrolysed by A mixture of 6 proteases	Anti-inflammatory properties and beneficial effects for treatment of arthritis	Ryu et al. 2010.
Marine	Giant squid (<i>Dosidicus gigas</i>) gelatine hydrolysed by seven proteases including Alcalase, Esperase and Neutrase	Improved ACE inhibition; potent cytotoxic effect against MCF-7 (human breast) and U87 (glioma) cancer cells, and antioxidant activity (FRAP and metal chelation)	Alemán et al. 2011.
Marine	Rockfish (<i>Sebastes hubbsi</i>) gelatine hydrolysed by Alcalase, Flavourzyme	Moderate free radical (DPPH, superoxide, hydroxyl, alkyl) scavenging properties, and ACE inhibition.	Kim et al. 2011.
Marine	Atlantic salmon skin collagen hydrolysed by Alcalase and papain	ACE inhibition	Gu et al. 2011.
Marine	Tuna dark muscle by-product hydrolysed by Papain or Protease XXIII	Dose-dependently inhibited breast cancer cells proliferation	Hsu et al. 2011.
Marine	Snow crab by-product by Protamex	Anticancer: potent inhibitory activity against the viability of lung, breast, colon, and prostate cancer cells	Doyen et al. 2011
Marine	Shrimp shell by-product hydrolysed by Cryotin enzyme	Anticancer: time-dependent inhibition of proliferation of Caco-2 (colon) and HepG2 (liver) cancer cells	Kannan et al. 2011
Marine	Purple sea urchin (<i>Strongylocentrotus nudus</i>) gonad hydrolysed by Neutrase, papain, pepsin, or trypsin	DPPH scavenging and FRAP antioxidant properties	Qin et al. 2011
Pea	Pea protein hydrolysates by alcalase	Inhibit renin activity	Li and Aluko 2010.
Pea	Pea protein hydrolysate	Antioxidant, anti-inflammatory & immunomodulatory properties	Ndiaye et al. 2011.
Pea	Enzymatic pea protein hydrolysates	Multifunctionality as ACE-inhibiting antioxidants	Humiski and Aluko 2007.
Pea	Enzymatic hydrolysate of protein isolate	Increased water solubility and amphiphilicity	Ribotta et al. 2012.
Peanut	Peanut hydrolysate reacted with xylose via Maillard reaction	Induced peptide degradation and crosslinking	Su et al. 2011.
Peanut	Hydrolysate of peanut protein isolate	Improved emulsifying properties of peanut protein isolate	Hu et al. 2011.
Hazelnut	Hydrolysed hazelnut proteins by trypsin, elastase and protease	Decreased allergenicity	Wigotzki et al. 2000.

Table 3 (continued)

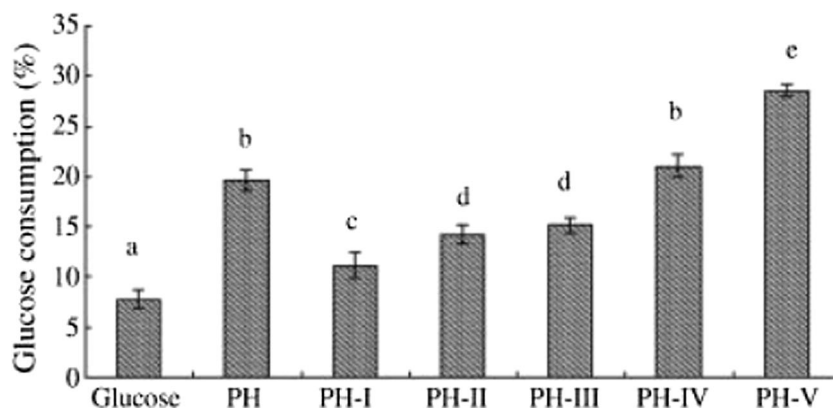
Source	Protein hydrolysate	Functionality	References
Hemp seed	Hemp seed protein hydrolysates	Potent renin inhibitor of renin and ACE activities.	Girgih et al. 2011.
Soybean	Soy protein hydrolysate by a multienzymes including trypsin, chymotrypsin, and peptidase	Improved digestibility of protein	Achouri and Zhang 2001.
Soybean	Soy proteins hydrolysed by proteases	Reduced allergenic potency	Yamanishi et al. 1996.
Soybean	Peptides from soy protein hydrolysis	Anti-inflammatory properties by decreasing ROS production, TNF- α , IL-6, IL-1 β , nuclear factor- κ B (NF- κ B) levels, and down regulation NO/PGE2 synthesis and inducible NOS/COX-2 expressions in activated macrophages	Dia et al. 2009; Gonzalez de Mejia and Dia 2009; Hernández-Ledesma et al. 2009.
Soybean	Soy protein hydrolysate	Preventing age-related chronic diseases	Wang and Gonzalez De Mejia 2005.
Soybean	Soy protein hydrolysates	Hypocholesterolemic and hypolipidemic properties	Aoyama et al. 2000; Hori et al. 2001.
Soybean	Soy protein hydrolysates	Hypocholesterolemic activity by binding bile acids and neutral sterols	Cho et al. 2007; Yang et al. 2007.
Soybean	Hydrolysate of soybeans	Anticancer via inhibiting the viability of cultured leukaemia cells	Wang et al. 2008.
Soybean	Hydrolysate of soy-protein isolate by trypsin, Alcalase or chymotrypsin.	Increased water solubility and amphiphilicity	Kim et al. 1990.
Rice	Hydrolysed by actinase	Decreased allergenicity	Watanabe et al. 1990a, b.
Rice	Rice protein hydrolysate	Immunomodulatory properties	Takahashi et al. 1994.
Rice	Limited enzymatic hydrolysis of rice endosperm protein	Increased water solubility and amphiphilicity	Paraman et al. 2007.
Wheat	Wheat protein hydrolysate	Immunomodulatory properties	Horiguchi et al. 2005.
Wheat	Hydrolysed by bromelain	Decreased allergenicity	Tanabe et al. 1996; Watanabe et al. 1994, 1995.
Wheat	Limited hydrolysis of wheat gluten by proteases	Increased water solubility and amphiphilicity, and improved foaming and emulsifying properties	Popineau et al. 2002.
Wheat	Wheat gluten hydrolysate	Increased solubility and surface hydrophobicity.	Wang et al. 2006a, b.
Quinoa seed	Quinoa seed protein hydrolysates	Multi-functionality as ACE-inhibiting antioxidants	Aluko and Monu 2003.
Rapeseed	Hydrolysate rapeseed protein isolates	Increased water solubility and amphiphilicity	Chabanon et al. 2007.
Bean	Bean hydrolysed with commercial protease (trypsin 250, Difco), with/without protease from <i>Bacillus</i> sp.	Increased digestibility	Dias et al. 2010.
Chickpea	Chickpea protein hydrolysates	Reduced antigenic activity	Clemente et al. 1999a, b.
Sunflower seed	Sunflower hydrolysate with high levels of branched-chain amino acids and low content of aromatic amino acids	Nutritional support for patients with hepatic failure	Bautista et al. 2000.
Zein	Zein hydrolysate with high levels of branched-chain amino acids and low content of aromatic amino acids	Nutritional support for patients with hepatic failure	Tanimoto et al. 1991.

exposing intact hydrophobic residues with a molecular weight >5000 Da (Gauthier et al. 1993). It is worth noting that extensive modification could lead to a lower availability (De Jongh and Broersen 2012) and that chemical modification may cause changes in allergenic response and status of toxicity and impaired nutrition. Thus, constant re-evaluation on safe use of modified proteins as novel food ingredient is required (Petersen et al. 1998; Bernstein et al. 2003).

Modification of Proteins in the Context of Food Matrices During Food Manufacturing

Foods contain proteins, carbohydrates, lipids, micronutrients and water as well as bioactives. These components in different food matrices and in various food formats can exhibit different physico-chemical attributes due to different chemical interactions in a food matrix. Food processing during food industrial manufacturing or during food service modifies the physical

Fig. 1 Reactivity of Maillard reaction based on glucose consumption by peanut hydrolysate (PH) and its peptide fractions with different molecular weights (Su et al. 2011): *PH-I*, *PH-II*, *PH-III*, *PH-IV* and *PH-V* represent fractions with molecular weight of >10, 5–10, 3–5, 1–3 and <1 kDa, respectively. Different letters above the columns indicate significant difference ($p < 0.05$)



and chemical properties of foods and consequently influences the release and uptake of nutrients in food matrix (Troncoso and Aguilera 2009). More specifically, proteins are consumed mostly in a specific food matrix, i.e. in a meal or a processed food, and digested within a digesta matrix containing different food components. Proteins interact with other proteins or non-protein components (i.e. large polymers carbohydrates, lipids and proteins or small molecules phytochemicals, flavour compounds, salts, gas and water). These interactions along with processing methods and digestion conditions affect protein digestion behaviour (Parada and Aguilera 2007; Lundin et al. 2008). Proteins can influence food shelf life and stability via their antioxidant and anti-microbial activity and ability to form networks as gas exchange barriers and stabilize emulsion-/foam-based foods (Del Rosario Moreira et al. 2011; Mendis et al. 2005). Proteins in a food matrix that also contains a reducing sugar may undergo desirable or undesirable functionality changes due to the Maillard reaction during food storage or processing (Le et al. 2011; McMahon et al. 2009). The combined use of proteins and other food components can provide beneficial synergies and novel food properties (Buchner et al. 2011; Del Rosario Moreira et al. 2011). It is critical to understand how proteins are modified in food matrices during food processing and in digesta matrices during transit in the digestive system. This knowledge provides the rationale for selecting protein resources and methods for protein ingredient isolation and modification, designing food formulation and creating food structures.

Interactions Between Proteins and Other Food Components Including Bioactives

Food components interact via physical mechanisms, e.g. adsorption, absorption, evaporation, drying and particle size reduction and/or chemical mechanisms, e.g. interactions between functional groups of food constituent molecules including proteins, lipids and carbohydrates or even between a food and its packaging materials. The text below summarizes the interactions that occur between macrocomponents, e.g.

protein–protein, protein–lipid or protein–carbohydrate, and between macro and microcomponents, e.g. protein–small molecules like natural or added formaldehyde, oxidizing and reducing agents (ascorbic acid, phenols and free radicals and flavouring compounds). Particular focus is placed on the interactions of protein with phenolics or dietary fibres, due to increasing interest in their inclusion in human diet (Scott et al. 2008; Arts and Hollman 2005; Scalbert and Williamson 2000) and due to the potential challenges associated with their anti-nutritional effects, their roles in protein digestibility and sensory issues during food development (Hughes et al. 1996; Jaeger et al. 2009; Sade 2009).

Protein–protein interactions clearly occur during processing and storage of foods, which is evident by the desirable or negative effects on foods, e.g. interaction between whey proteins and casein micelles during heating could lead to desirable yield increase for cheese making but is undesirable for fluid milk production (Corredig and Dalgleish 1999). Protein–protein interactions present low stability challenges related to product hardening and syneresis of water for protein-rich foods (Dalgleish and Hunt 1995). Understanding of such interactions would support the rational design of processed foods. The polypeptide chains in most proteins occur in a defined structure, i.e. as helices, sheets and turns (Belitz et al. 2009). In addition to amide linkages, protein conformation is maintained by hydrophobic forces, hydrogen bonding, van der Waals forces, electrostatic interactions, ion bridging or covalent bonds like disulphide or lysine-alanine bonds (Dalgleish and Hunt 1995). Processing influences protein–protein interactions and ultimately food microstructures and textures. Protein–protein interactions can be induced by the potential covalent bonds between amino acids on different proteins like those in bread dough (Li and Lee 2000; Sun-Waterhouse et al. 2013a). When blending various proteins in a food system, their compatibility or incompatibility lead to different microstructures under different processing conditions, e.g. heat- or high-pressure-induced interactions of soy proteins (Utsumi and Kinsella 1985; Molina et al. 2002) and wheat proteins (Li and Lee 1996). This provides opportunities

to modulate the kinetics of aggregation or alter the reactive sites on the proteins (both existing and newly generated).

Protein–carbohydrate interactions are clearly evident by the Maillard reaction. The amino groups of most proteins are involved in peptide linkages; thus, the Maillard reaction occurs only at the free amino groups such as the N-terminal amino group of Glu, Asp and Lys (Le et al. 2011). Conjugation between proteins and polysaccharides improves the structural stability and emulsifying properties of proteins (Kato et al. 1993). Proteins can be crosslinked via covalent bonds to food components containing reactive moieties like sulfhydryl, amine or carbohydrate functional groups during lipid peroxidation and Maillard reaction (Singh 1991; Friedman 1999). Protein binds to the non-starch polysaccharides via Oglycosidic linkages or diferulate crosslinks due to oxidation (which can impair protein digestion) (Fry 1988; Damodaran 1996). Protein also interacts with starch in plant foods such as sorghum and corn and in protein-rich processed foods like yoghurt and meat products (Marshall and Chrastil 1992; Rooney and Pflugfelder 1986), causing functionality changes of both starch and protein, e.g. decreased protein digestibility due to a close association between starch and protein (Oria et al. 1995) as well as decreased starch gelatinisation and digestibility (Rooney and Pflugfelder 1986). While raising dietary fibre content in foods is a convenient way to increase fibre consumption for wellness, it is worth noting that interactions between proteins and some fibre polysaccharides could cause undesirable changes in food attributes such as phase separation and decreased foam stability, and such interactions may be dynamic over the process of food processing, storage, ingestion and digestion (Bhat et al. 2006; Mirhosseini and Amid 2013). In-depth investigations are required on different native and modified proteins used for food formulation.

Protein–lipid interactions exist and an obvious example is the emulsion for preparing cheese, ice cream, cream, milk, mayonnaise, dough and sausages (Blond et al. 1988; Le Meste and Davidou 1995; Sivam et al. 2012; Sun-Waterhouse et al. 2013a). Proteins and phospholipids are both surface-active and thus often co-exist in the same ingredient or finished food systems such as milk and egg yolk. Phospholipids can enhance protein's emulsifying capacity via complexation (Hirotsuka et al. 1984; Nakamura et al. 1988; Mine et al. 1994).

Protein–small molecule interactions exist and should not be ignored. Understanding of the interaction between protein and flavour compounds is important for the development of protein-rich foods with consumer acceptance. Most proteins do not contribute to flavour directly but can influence taste sensation through their binding or complexation with small flavour compounds and other food components that are released from foods during mastication (Martens et al. 1987; O'Keefe et al. 1991). Processing conditions including

temperature and pH affect the binding of flavour compounds to proteins as well as the conformation and total surface area of proteins (Kinsella et al. 1985; Andriot et al. 2000; Heng et al. 2004). Generation of free radicals is inevitable during food processing and storage due to stress of heat and shear. *Interactions between free radicals and proteins* are possible due to their chemical structure and reactivity. For example, free radicals are involved in protein denaturation and aggregation and consequently some important amino acids like Cys, Lys, His and Met are lost (Howell and Saeed 1999). *Formaldehyde* either naturally occurs in foods or is added to influence protein conformation and improve food functional properties, e.g. enhancing stability of milk and cheese and wheat dough development (Gerrard et al. 2003; Holt et al. 1978). Essentially, formaldehyde is crosslinking agent and can react bifunctionally to create crosslinks with the side chains of Lys, Cys, Tyr, His, Try and Arg residues via methylene bridges and disulphide, hydrophobic and hydrogen bonds (Sikorski et al. 1976). Formaldehyde reacts with thiols faster than with amines and decreases the sulfhydryl groups and hydrophobicity as well as increases the toughness of fish muscles (Lewin 1956; Sotelo et al. 1995). *Ascorbic acid* (vitamin C) is either naturally occurs in foods or is added as a food additive, i.e. an antioxidant or vitamin supplement to improve food shelf-life, nutritional value and gelling properties (Bauernfeind 1982; Fitchett and Frazier 1986; Nishimura et al. 1992). Ascorbic acid complexes with proteins (Fleming and Bensch 1983) and also reacts with amino groups leading to browning/Maillard reactions (Lewin 1974; Eiserich and Shibamoto 1994; Alaiz et al. 1997).

Polyphenol–protein interactions are mainly due to hydrophobic interactions between amino acids side chains and polyphenol aromatic rings and hydrogen bonding via between –OH groups of polyphenols and protein chain, although covalent bonding is also possible (Wollgast and Anklam 2000; Joiner et al. 2004). These interactions cause full or partial unfolding of protein chains, leading to soluble or insoluble complexes (Rawel et al. 2003; Richard et al. 2006) and proteins with different extractability and size (Fig. 2, Sun-Waterhouse et al. 2011). The formation of polyphenol–protein complex decreases the digestibility, bioavailability and other functional properties of both proteins and polyphenols as well as the activity of some digestive enzymes (Griffiths and Moseley 1980; Ahmed et al. 1991; Haslam et al. 1991; Arts et al. 2002; Papadopoulou et al. 2005; de Toledo et al. 2013). Not all proteins are equally involved in forming hazes, and proteins rich in proline likely have strong haze-forming activity (Asano et al. 1982). The extent of the interactions depends on the structure of protein and polyphenol molecules, their ratio and surrounding environment (i.e. pH, ionic strength, temperature and composition) (Riihimäki et al. 2008). Soluble protein–tannin complexes can grow until reaching a size at which the complexes become soluble, or they may undergo

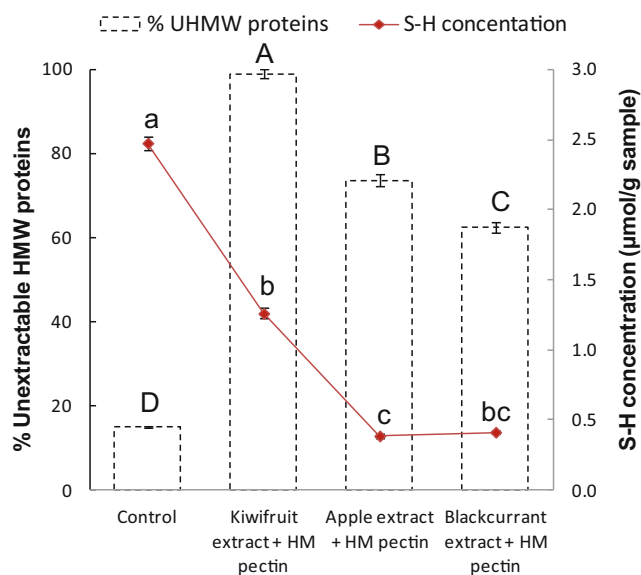


Fig. 2 The %Unextractable HMW proteins and free S-H content to evaluate the protein size distribution of the control and polyphenol-fortified breads (Sun-Waterhouse et al. 2011). Different uppercase (A–D) and lowercase (a–c) letters indicate the %Unextractable HMW and free S-H content statistically significant differences at $p < 0.05$, respectively

changes such as aggregation and precipitation (Luck et al. 1994). Oxidative conjugation of protein–flavonoids may present an approach to improve the properties of proteins and retain the antioxidant property of flavonoids such as Laccase-catalysed protein–flavonoid conjugates (Kim and Cavaco-Paulo 2012). Pascal et al. (2007) proposed multi-stage mechanisms of interaction between human salivary proline-rich protein and polyphenols, i.e. flavan-3-ol monomer epigallocatechin-3-gallate (EGCG): At low protein concentration, the EGCG/protein ratio determines the three-step protein–polyphenol agglomeration, whereas, at high protein concentration, the affinity of EGCG to proteins occurs well ahead of the saturation of interaction sites; thus, aggregate forms at a much lower EGCG/protein ratio. Salt addition, pH change or temperature change all influence the interactions (Wang et al. 2007a, b; Shpigelman et al. 2010; Staszewski et al. 2011). The polyphenol–human salivary protein interactions contribute to the perception of food astringency (Kallithraka et al. 2001; Jaeger et al. 2009).

Interactions of polyphenols with enzymatic proteins are also possible, which would follow the mechanisms similar to those of the non-enzyme proteins. Polyphenol–enzyme interactions alter enzyme configuration causing formation of insoluble aggregates, enzyme denaturation and decreased catalytic activity of enzymes such as salivary α -amylase, tyrosinase, peroxidase, proteases, pepsin, trypsin, glycosidases, lipase, decarboxylase, squalene epoxidase and ribonuclease (Griffiths 1984; Nakahara et al. 1993; Bertoldi et al. 2001; Rohn et al. 2002; Huang et al. 2004; McDougall and Stewart

2005; McDougall et al. 2005). Interaction of polyphenols and enzyme affect the functional properties of both components, which depend on pH, temperature, type and concentration of polyphenols and proteins (including the number and position of –OH group and the type and position of glycosyl) (Luck et al. 1994; van der Burg-Koorevaar et al. 2011; Yang et al. 2011). Polyphenols can exert anti-nutritional effect on digestive enzymes despite the fact that polyphenols themselves are natural antioxidants with various health benefits (Tagliazucchi et al. 2005; Piparo et al. 2008; de Toledo et al. 2013). Furthermore, *polyphenols can interact with biological proteins directly* leading to desirable or undesirable physiological changes, e.g. promoting the constriction of organic tissues which can be used to control haemorrhage and blood pressure and inhibit mucous secretion (Corder et al. 2001; Okamoto 2006; Perez-Vizcaino et al. 2009) and detrimental effects on the digestive tract mucosa (Luck et al. 1994).

Few studies investigated the food matrix effect on the digestibility of food components and these studies mostly employed in vitro methods. For example, the bioaccessibility of carotenoids varied with food matrix (Reboul et al. 2006). The composition of food matrix influences the stability and digestibility of the polyphenol components. The soluble fraction and the polyunsaturated fatty acid-rich lipid fraction from carob flour protect phenolics against pH changes and enzymatic activities during digestion, and such protection depends on the type of phenolics (Ortega et al. 2011). Microcomponents co-existing in foods such as iron and bile acids may also affect the bioaccessibility of phenolics (Dongowski 2007). The presence of carbohydrates like sugars in the ingested foods could increase the bioavailability of flavonols from cocoa (Schramm et al. 2003), catechin from tea (Peters et al. 2010) and flavan-3-ols from chocolate products (Neilson et al. 2009). Contradictory findings were reported on the effect of milk or milk proteins on the antioxidant activities of tea and coffee polyphenols; adding milk, sugar, or milk plus sugar to tea stabilized or increased the antioxidant activity of polyphenols, even though their radical scavenging activity decreased (Sharma et al. 2008), whereas whey protein with >50 % total solids decreased the antioxidant activity of green tea infusions (von Staszewski et al. 2011). Therefore, there is a need to investigate the differences in protein digestibility and functional properties of final consumer foods enriched with proteins and target bioactives. Approaches that can reduce the anti-nutritional/detrimental effects of foods containing polyphenols, enzymes and microorganisms include tailoring food matrices through the inclusion of some biopolymers such as salivary proline-rich proteins (Naz et al. 2011) or pectin (Table 4) and other fibre gum (Gonçalves et al. 2011; Sun-Waterhouse et al. 2011, 2013c) or employing encapsulation technologies such as co-extrusion and spray drying (Sun-Waterhouse et al. 2012, 2013b; Shinde et al. 2013). So far, there still are few detailed study that rationalized the

Table 4 Individual anthocyanin content in blackcurrant extract-enhanced yoghurts (Sun-Waterhouse et al. 2013c)

Yoghurt formulation		Delphinidin-3- <i>o</i> -glucoside (mg/200 g yoghurt)	Delphinidin-3- <i>o</i> -rutinoside (mg/200 g yoghurt)	Cyanidin-3- <i>o</i> -glucoside (mg/200 g yoghurt)	Cyanidin-3- <i>o</i> -rutinoside (mg/200 g yoghurt)
Low methoxyl pectin	Prefermentation	11.25±0.06 ^{Da}	44.82±0.10 ^{Ba}	26.60±0.09 ^{Ca}	175.6±0.1 ^{Aa}
	Postfermentation	9.10±0.06 ^{Dc}	22.75±0.08 ^{Bc}	9.37±0.05 ^{Cc}	46.23±0.09 ^{Ac}
High methoxyl pectin	Prefermentation	10.89±0.06 ^{Db}	43.35±0.06 ^{Bb}	25.73±0.07 ^{Cb}	169.8±0.1 ^{Ab}
	Postfermentation	7.82±0.05 ^{Dd}	19.56±0.03 ^{Bd}	8.06±0.05 ^{Cd}	39.73±0.07 ^{Ad}

Data expressed as mean±standard deviation of replicate measurements. Different uppercase superscript letters (within the same entire row) indicate statistically significant differences at $p<0.05$. Different lowercase superscript letters (within the same entire column) indicate statistically significant differences at $p<0.05$

blending of different native and/or modified proteins from different sources based on the protein digestibility and food processability and nutritional/functional properties for humans. Future research should be directed towards this area.

Proteins experience various intermolecular and intramolecular interactions in a food matrix. Thus, it is important to tailor food formulation and processing to modulate potential chemical and physical reactions such that food quality, protein digestibility and bioactive delivery to humans can be maximized. Various protein gels with different microstructural characteristics, such as fine-stranded gels (composed of more or less flexible linear strands with a regular and elastic network of high resistance to rupture) and particulate gels (composed of large and almost spherical aggregates with low elastic behaviour and lower rupture resistance), can be produced through controlling ionic strength, pH, heating, pressure and enzymes (Dalgleish and Hunt 1995). Microstructure of these protein gels greatly influences protein digestion and bioactive release, e.g. particulate gels with large porosity and a high-pore interconnectivity, would facilitate a greater rate of digestion than fine stranded gel (Maltais et al. 2009). Purwanti et al. (2010) proposed the creation of desirable structures from concentrated globular proteins (Fig. 3), i.e. structural elements as domains in the form of particles, fibres etc with sizes ranging from nanometres to millimetres. This proposed model clearly illustrates the food matrix effects, via interactions between the structure elements as well as between the elements and continuous phase. Structural elements prepared at low protein concentrations are proposed to act as structural breakers in a high-protein matrix (Fig. 3a). These structural breakers in sufficient amounts would retard and reduce protein hardening. In comparison, structural elements containing a high protein content could serve as building blocks in a matrix with no or low protein (Fig. 3b). The storage stability and overall sensory attributes of the food system won't be affected by an increase in the strength of these elements, as long as the continuous phase remains constant. This proposal of structural elements, i.e. protein particles in a protein/non-protein matrix, may help with optimizing food component interactions and selecting appropriate

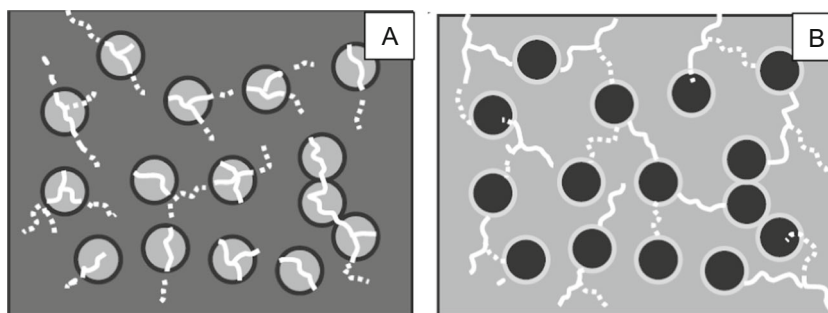
processing methods, e.g. microparticulation (Onwulata et al. 2002) or manipulation of biopolymer phase separation (Norton and Frith 2001).

Processing Effects

Food processing under the conditions of food manufacturing and physiological digestion involve heat, pH, pressure and shear treatments, dehydration, microbial process, enzymatic reactions and exposure to ionic environment or oxidizing agents. Thus, proteins in a food matrix system would undergo further “in situ” modification through interacting with other co-existing food components during processing, storage, ingestion and digestion, inducing changes in protein structure, reactivity and functional properties (Gardner 1984; van Beresteijn et al. 1994; Thomas et al. 2004; Pushparaj and Urooj 2011). Food processing modifies the profile of the bioactive peptides produced, forming peptide sequences with different digestibility that do not occur naturally in the precursor protein ingredients (Gardner 1984). Processing can reduce the content and/or allergenic activity of protein allergens (Kilshaw et al. 1982). Thus, food processing provides opportunities to further maximize the digestibility and nutritional value of food proteins (Meisel and Schlimme 1990), although effort is still required to avoid unfavorably changes of proteins, e.g. conversion of L- to D-amino acids and generation of indigestible peptide bonds (Liardon and Ledermann 1986; Gardner 1984).

Heating is one of the most commonly used food processing methods, which can cause positive or negative changes in the nutritional and/or functional properties of proteins, e.g. heat-induced protein denaturation may not only improve protein water binding and emulsification but also reduce protein solubility due to aggregation or coagulation (Boye et al. 1997; Finot 1997). Heating accelerates the Maillard reaction by which the functionality of proteins in foods is modified (Gerrard et al. 2002). Heat-induced protein denaturation is important for food manufacturing, but presents limitations for globular proteins due to texture issues (Schmitt et al. 2007) especially at a high protein concentration. Above the

Fig. 3 Overview of structural elements low in proteins as structural breakers in a high-protein matrix (a) and structural elements rich in proteins as structural builders in a low-protein matrix (b) (Purwanti et al. 2010)



critical concentration, protein gelation often occurs below the denaturation temperature (Hongprabhas and Barbut 1997). For *milk proteins*, heating milk enhances protein resistance to in vitro digestion due to the structural changes caused by denaturation and aggregation of whey and casein proteins and the disulphide bonds in whey proteins (Dupont et al. 2010). Heating induces aggregation of whey proteins especially β -lactoglobulin as a function of pH, concentration, temperature and ionic strength because of protein unfolding (Schokker et al. 2000). For *meat proteins*, cooking affects the susceptibility of myofibrillar protein to proteases, i.e. increases or decreases the reaction rates depending on the type of protease, time and temperature, because cooking induces protein carbonylation and aggregation (Sante-Lhoutellier et al. 2008). Cooking of meat also promotes protein and lipid oxidation (Badiani et al. 2002; Sante-Lhoutellier et al. 2008). For *plant proteins*, cooking may change their digestibility differently depending on the type of proteins, e.g. cooking reduces sorghum protein digestibility as protein oligomers and polymers crosslinked via disulphide bonds form (Belton and Taylor 2004), but does not change maize protein digestibility (Hamaker et al. 1986). Cooking sorghum with reducing agents can improve protein digestibility (Hamaker et al. 1986; Arbab and El Tinay 1997). Thermal treatment affects both *intact proteins* and *protein enzymatic hydrolysates*. Cui et al (2009) reported that increasing heating temperature led to decreased SH group but increased S-S groups of intact chicken proteins (Fig. 4), and after heating, the chicken protein hydrolysates by Alcalase had lower amounts of the protein fractions with >10,000, 3500–2640 and <2640 Da molecular weight.

Drying is a commonly used in food processing, and the characteristics of the dried product are determined by the nature of food and the conditions of drying process (Mirhosseini and Amid 2013). Various drying methods are used to dry protein foods, including evaporation with/without atomization (e.g. spray drying), sublimation (e.g. freeze drying and spray freeze drying) and precipitation (e.g. supercritical fluid technology) (Abdul-Fattah et al. 2007). These drying methods present different stresses that damage native protein structure to different degrees, e.g. heating and shearing stresses in spray drying and freezing stress in freeze drying (Abdul-

Fattah et al. 2007). Spray drying and freeze drying are often used for drying protein solutions. Spray drying is a cost-effective drying method that has the advantage of being a rapid single step process (Maa and Prestrelski 2000). Freeze drying is unique in its drying step after freezing, i.e. the material is dried as a solid which facilitates stability although this method requires a longer process thus is less cost-effective (Seager 1998). Drying leads to amorphous, crystalline/liquid crystalline powders or their mixture that possess different physico-chemical properties and stability (Lu and Zograf 1998; Yu 2001; Lechuga-Ballesteros et al. 2003). Ovalbumin powders produced by spray drying or freeze drying exhibited different emulsifying and foaming properties upon reconstitution (Kitabatake et al. 1989). The water-holding capacity of protein-rich foods varies with the hydrophilic–hydrophobic balance of amino acids in the protein and the co-existing lipid and carbohydrate contents (Chou and Morr 1979). Thermal denaturation of proteins occurs much more readily in the presence of water (Zaks 1992). Freezing as the first step of freeze drying causes destabilizing stresses, and the stress, due to the “dehydration effect” after water removal from protein via ice formation, results in decreased hydrophobic interactions (Tang and Pikal 2004). Varied freezing rates result in different stresses to protein-rich foods depending on the nature of protein, and slow freezing, as opposed to rapid freezing in

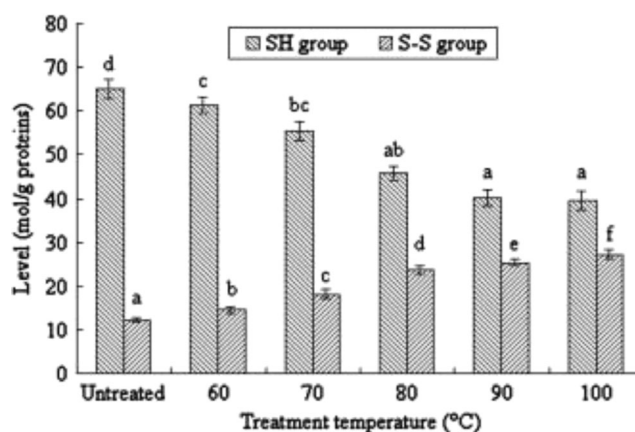


Fig. 4 Effect of temperature on the levels of SH and S-S groups in chicken proteins (Cui et al. 2009). Different letters above the columns represent significantly different $p < 0.05$

liquid nitrogen or dry ice-acetone bath, may damage more proteins in unstable systems with a phase separation tendency (Heller et al. 1999; Tang and Pikal 2004). Protein concentration is an important parameter for consideration during freezing, since a highly concentrated protein solution leads to large ionic strengths, pH shifts and increased protein–protein interactions (promoting protein aggregation and degradation) (Pikal et al. 1991). Furthermore, selecting an appropriate formulation for protein-rich food is as crucial as choosing a proper drying method because the co-existing food components would impart different interactions with proteins, e.g. chelation or not directly exerted different impact by the stabilizers on the protein surface coverage (Millqvist-Fureby et al. 1999a, b).

Natural or controlled fermentation has been long used as an effective and low-cost process to preserve foods while retaining or modifying their nutritional properties such as cheese, yoghurt, tofu and bread (Parveen and Hafiz 2003). Many kinds of soy sauce are made through fermentation of a paste of boiled soybeans, roasted grain, brine, and *Aspergillus oryzae* or *Aspergillus sojae* moulds. During this process, the proteins in the paste are broken down into peptides (Shurtleff and Aoyagi 2007). Beans after fermentation are also easier to digest by humans (Katz 2003). Fermentation can lead to the loss of reactive thiols thus changing the palatability of sorghum flour products and the reduction of adverse antigenic/allergenic reactions of foods especially dairy foods via proteolysis (Pescuma et al. 2011; Chobert 2012). Fermentation of a mixture of plant materials may offer advantages over a single plant material in terms of crude protein, amino acid profile and antioxidant content (Oyarekua 2009). While fermentation is widely believed to enable the nutrients (e.g. micronutrients and amino acids) and bioactives (e.g. polyphenols) in foods to become more readily available (Fig. 5; Sun-Waterhouse et al. 2013a; Odumodu 2007, 2008), contradictory findings were reported based on in vivo rat trials, e.g. fermentation affected the protein quality negatively, as the biological value and net protein utilization of the fermented blends were worse than the unfermented blend (Odumodu 2010). Thus, human studies on various fermented protein-rich foods are required.

Germination or seed sprouting is an inexpensive technique for improving the utilization and nutritional value of proteins, including increased protein digestibility and availability of seeds and legume proteins through the relocation of stored proteins (but without some anti-nutrients like enzyme inhibitors) to the growing sprout (Maeda et al. 1991; Pushparaj and Urooj 2011). Moreover, germination offers an approach to improve nutritional and physical properties of pulses, cereals and other grains, e.g. increase dramatically nutrient and bioactive contents including dietary fibre, phenolic acids, minerals and some special bioactives like gamma amino butyric acid (Charoenthaikij et al. 2009; Kaosa-ard and Songsermpong 2012). Germinated brown rice was found to

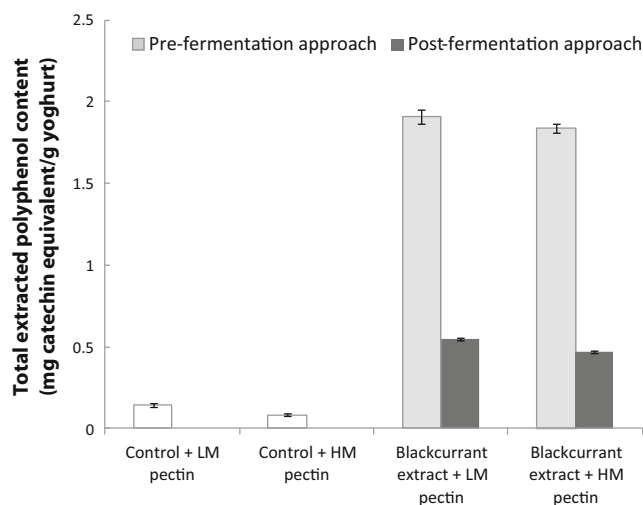


Fig. 5 Total extracted polyphenol contents of the control and blackcurrant polyphenol extract-enhanced yoghurts (Sun-Waterhouse et al. 2013a). *LM* and *HM* refer to low and high methoxyl pectins, respectively. *Pre* or *postfermentation approach* refers to adding blackcurrant extract to yoghurt before or after fermentation

contain a significant amount of brain health-promoting gamma amino butyric acid, and the content of this bioactive increased dramatically with soaking time (Kaosa-ard and Songsermpong 2012). Germination can also increase the protein and phenolic content through fortifying proteins and phenolics from other sources (Randhir et al. 2004), e.g. increase the tannin content of pearl millet spout (Pushparaj and Urooj 2011). Innovative technologies are available for controlled partial germination such as the Pargem® process that enables industrial-scale germination and produces food products with improved taste (i.e. desirable sweet note), reduced anti-nutrient content and increased micronutrients (<http://www.buhlergroup.com/global/en/products/pargem-bmpa.htm#UpmPAYdzdy4>).

High-pressure processing (HPP) is safe and preservative-free non-thermal food preservation technology that offers opportunities to restructure food proteins through tailoring the process parameters such as pressure, time, temperature, protein concentration, pH and food matrix composition like salts, with advantages of reduced nutritional and flavour loss and shortened processing time (Rastogi et al. 2007). HPP compresses protein internal cavities, breaks non-covalent interactions within proteins and reforms intra and intermolecular bonds, causing protein denaturation, aggregation or gelation (Cekko 1991; Tewari 2007). HPP does not affect covalent bonds such as cross linkages within macromolecules except for the disulphide bonds in proteins (Heremans 2001). Protein tertiary and secondary structures tended to change significantly at pressures >200 MPa (Tauscher 1995). Low pressures, e.g. 100–300 MPa, often cause reversible changes including dissociation of protein–protein complexes, binding of ligands and conformational changes, whereas pressures >500 MPa

mostly lead to irreversible denaturation (Heremans et al. 1997; Rastogi et al. 2007). Ultra-high pressure (UHP) processing of milk presents an alternative way to extend shelf-life with additional quality (Messens et al. 1997). Table 5 lists the published original research in which HPP was used to induce changes in proteins from different sources. HPP has been applied to milk proteins the most. For *milk proteins*, HPP can increase milk dynamic viscosity but reduce milk turbidity and lightness (Shibauchi et al. 1992). High pressure ≥ 150 MPa destabilizes casein micelles and reduces casein micelle size of reconstituted skim milk at 20 °C (Shibauchi

et al. 1992). At neutral and alkaline pHs, the reactivity of SH groups is increased and HPP induces aggregation and gelation of whey proteins (Van Camp and Huyghebaert 1995a, b). β -Lactoglobulin is more susceptible to pressure than bovine serum albumin and α -lactalbumin (Hayashi et al. 1987; Nakamura et al. 1993a, b) and when treated at 400–800-MPa digestion by pepsin is promoted (Zeece et al. 2008). For *meat proteins*, HPP can tenderize meat, although denaturation, aggregation and even loss of protein structure are possible at high pressures, e.g. myoglobin at 2,400 MPa (Homma et al. 1995; Cheah and Ledward 1996). The minimal pressure

Table 5 Modifying milk, egg, meat and plant proteins through high-pressure processing

Protein source	Dairy (whole milk, whey and casein)	Egg (whole egg, egg white and egg yolk)	Meat and fish	Plant (soy, wheat, red kidney bean etc)
Original research	Bonomi et al. 2000, 2003. Buffa et al. 2001. Chicón et al. 2006. Denda and Hayashi 1992. Desobry-Banon et al. 1994. Dumay et al. 1994. Galazka et al. 1995, 1996. García-Amezquita et al. 2009. García-Graells et al. 2000. García-Risco et al. 1998, 2000. Gervilla et al. 2001. Hayashi et al. 1987. Hayakawa et al. 1992. Hite 1899. Huppertz et al. 2003, 2004 Johnston and Darcy 2000. Johnston et al. 1992, 1993. Kim et al. 2008. Knudsen et al. 2002. Lee et al. 1996. López-Fandiño et al. 1996. López-Fandiño and Olano 1998. Maynard et al. 1998. Messens et al. 2000. Moltó-Puigmartí et al. 2011. Mussa and Ramaswamy 1997. Nakamura et al. 1993a, b. Ohmiya et al. 1989. Okamoto et al. 1991. Olsen et al. 2003. Otte et al. 1997. Regnault et al. 2004. Shibauchi et al. 1992. Shibauchi et al. 1992. Schrader and Buchheim 1998. Schrader et al. 1997. Sierra et al. 2000. Stapelfeldt et al. 1996. Van Camp and Huyghebaert 1995a, b. Van Camp et al. 1996. Van Willige and Fitzgerald 1995. Ye et al. 2004. Zasyarkin et al. 1996. Zobrist et al. 2005.	Doi et al. 1991. Hamid-Samimi et al. 1984. Hamid-Samimi and Swartzel 1984. Hayashi et al. 1989. Herald et al. 1989. Hoppe et al. 2013. Iametti et al. 1998, 1999. Juliano and Bilbao 2012. Kovacs-Nolan et al. 2000, 2005. Li-Chan et al. 1995. Lopez-Exposito et al. 2008. Ma et al. 1993. Martos et al. 2010. Messens et al. 1997. Miguel and Aleixandre 2006. Miguel et al. 2007. Mine 1995, 1997, 2002, 2007. Ngarize et al. 2004, 2005. Okamoto et al. 1990. Ponce et al. 1998a, b, c. Quiros et al. 2007. Rajan et al. 2006. Richwin et al. 1992. Shen et al. 2010. Singh and Ramaswamy 2013. Smith et al. 2000. Van der Plancken et al. 2004, 2005, 2007a, b. Vogtt and Winter 2005. Wu et al. 2010. Yoshino et al. 2004. You et al. 2010a, b.	Ananth et al. 1998. Ashie et al. 1999. Cheah and Ledward 1996. Defaye and Ledward 1995. Defaye et al. 1995. Homma et al. 1995. Huang et al. 1999. Patterson and Kilpatrick 1998. Shigehisa et al. 1991. Suzuki et al. 1994. Yamamoto et al. 1992. Yuste et al. 1998.	Anonymous et al. 2011. Apichartsrangkoon et al. 1998. Kajiyama et al. 1995. Kieffer et al. 2007. Matsumoto and Hayashi 1990. Okamoto et al. 1990. Liu et al. 2013. Wang et al. 2011. Yin et al. 2008.

required to induce such changes varies with the type of meat protein and can be as low as 140 MPa (Yamamoto et al. 1992; Defaye and Ledward 1995). For *egg proteins*, HPP-treated egg white can gel but the natural flavour and nutritional value of egg white are retained (Hayashi et al. 1989), e.g. it partially coagulates at 500 MPa but forms strong self-supporting gels at ≥ 600 MPa (Okamoto et al. 1990). The conformation of ovalbumin remains fairly stable at 400 MPa (Hayakawa et al. 1992) and even up to 600 MPa, its hydrogen bonds are not destabilized (Doi et al. 1991). The extent of HPP-induced ovalbumin denaturation is much less than the denaturation caused by either heat or chemicals (Hayashi et al. 1989). For *soy proteins*, gelation is possible when a pressure not less than 300 MPa is applied for 10–30 min (Matsumoto and Hayashi 1990). HPP can be used for tofu making. The HPP-tofu gels (300 MPa for 10 min) have a comparable hardness to the heat-set gels (Kajiyama et al. 1995). Soy milk is still in liquid form and possesses improved emulsifying capacity and stability at < 500 MPa for 10 min, but can change to a solid state at 500 MPa for 30 min (Kajiyama et al. 1995). HPP can modify the structures and increase the surface hydrophobicity of *other plant proteins* such as wheat and legume proteins, to achieve improved functionality (Kieffer et al. 2007; Liu et al. 2013). Liu et al. (2013) reported that HPP increased the yield of crude red kidney bean extract but decreased its haemagglutination activity, altering phytohaemagglutinin secondary structures (Fig. 6), e.g. an intense IR band around 1635 cm^{-1} (assigned to intermolecular parallel β -sheet structures) occurred to HPP-treated samples due to protein unfolding, compared with the native sample. It is of interest that HPP may facilitate the release of bioactive peptides and also increase protein digestibility, e.g. egg white proteins ovalbumin, ovotransferrin, and lysozyme treated at 400–800 MPa and 4°C for 5 min exhibited *increased pepsin digestibility* (Hoppe et al. 2013).

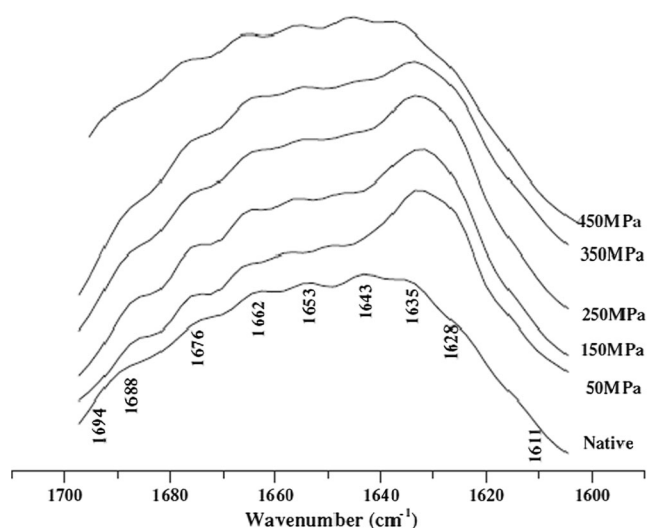


Fig. 6 Deconvoluted FTIR spectra of native (0.1 MPa) and high hydrostatic pressure-treated red kidney bean homogenates (Liu et al. 2013)

Pulsed-electric field (PEF) treatment is a non-thermal food preservation technique that ruptures microbial cell membranes, to achieve microbial inactivation for strains including heat-resistant *Escherichia coli* (Wouters et al. 1999; Manas et al. 2001). Depending on the strength applied, PEF possibly induced an elevated protein dielectric constant causing polypeptide unfolding, changes in charge distribution and hydrophobicity and dissociation of non-covalent bonding between protein subunits, thus subsequently loosening protein secondary and tertiary structures (Kinsella et al. 1994; Barsotti et al. 2000). As a result, some protein aggregation may occur due to increased exposure of hydrophobic and thiol groups, and inhibition of enzyme catalytic activity is also possible due to the increased failure in binding substrate (reviewed by Zhao et al. 2013). The in-depth mechanism of PEF treatment on proteins currently remains unknown, although less influence on protein by PEF than thermal pasteurization is confirmed (Marco-Molés et al. 2011), e.g. thermal treatment caused dialysed egg white to gel with markedly different mechanical properties and water retention characteristics, while controlled PEF (200 pulses, 30 kV/cm, 80 nF) did not cause notable changes in the proteins but only slightly reduced gelling properties (Fernández-Díaz et al. 2000). Zhao et al. (2013) proposed the combined use of PEF and other processing aids such as addition of anti-microbial substance, moderate heat and pH to retain maximal protein quality. Quite a few studies reported PEF's minimal impact on plant-based small molecule compounds including phytochemicals like phenolic compounds (reviewed by Odriozola-Serrano et al. 2013), which further indicates the suitability of PEF for protein-rich foods fortified with health-promoting phytochemicals.

Extrusion is used to texturise protein products and allows the creation of high-protein extruded products via incorporating a large amount of exogenous proteins (Bohner and Reynolds 2006; Allen et al. 2006). Extrusion processing has been used by the food industry to manufacture pastas, puffed snacks, candies and meat analogues (Heldman and Hartel 1997). In the past, protein extrusion has failed to effectively create products with desirable textural quality, except for some whey and soy protein products (Arêas 1992; Liu and Hsieh 2007; Onwulata et al. 2003, 2010). Extruding protein alone at high concentrations such as whey proteins is challenging; thus, milk protein concentrates are mostly blended with starch and fibre base to produce extruded/puffed snacks with desirable texture (Manoi and Rizvi 2008). Dairy proteins can likely be added to conventional extruded starch-based snacks to improve product nutritional value; however, this approach may lead to non-expandable structure and tough texture due to the increase of the number of crosslinking sites (Martinez-Serna and Villota 1992; Allen et al. 2006), thus requiring modification of extrusion methods (Enrione et al. 2012). Plant proteins, singly or in combination, can be processed mostly

via thermoplastic extrusion technology into products with fibrous structures formed by protein thin filaments or microfibrils (Hu et al. 1995; Liu and Hsieh 2007). Temperature is the main factor that affects the nutritional quality of plant proteins such as wheat, rice and soya bean proteins (Hu et al. 1995).

Microparticulation is a technique that is used to generate protein particles with a broader range of sizes. This technique is mostly utilized along with other processing methods such as extrusion (Queguiner et al. 1992). Several methods for microparticulation have been used to confer a fat-like mouthfeel including scraped-surface heat exchangers, high shear homogenizer, microfluidizers, high-pressure homogenizer (690 MPa for 5 min at room temperature), ultra-high pressure (UHP) treatment temperature (Lee et al. 2006), double emulsification (Surh et al. 2007), extrusion or even dissolving a protein gel into alkaline solution with/without agitation (Queguiner et al. 1992; Jost 1993; Onwulata et al. 2002; Lee et al. 2006; Yoo et al. 2007).

Protein crosslinking, either non-enzymatic or enzymatic crosslinking, provides specific and natural approaches for modifying food structure (Gerrard 2002). *Non-enzymatic crosslinking* of proteins affects gelling and textural properties (Utsumi and Kinsella 1985), heat stability (Singh 1991), emulsifying and foaming capacity (Lásztity et al. 1998) and thermo-rheological properties (Lee et al. 1997). In general, animal proteins such as bovine serum albumin and α -lactalbumin are more susceptible to the formation of lysinoalanine crosslinks than plant proteins like soy proteins, and the reaction rates increase with elevated pH, time and temperature (Whitaker and Feeney 1983; Chung et al. 1986). Severe heat treatment of pure proteins egg albumin, casein, lysozyme, muscle proteins or protein-rich foods with a low carbohydrate content result in isopeptide crosslinks (Otterburn et al. 1977). Disulphide crosslinks can be induced by mechanical mixing, heating or enzymatic catalysis through oxidizing the sulfhydryl groups on cysteine residues in a protein (Utsumi and Kinsella 1985). *Enzymatic crosslinking* is more widely used than non-enzymatic crosslinking due to the desirable characteristics, i.e. specificity and mildness of enzymatic reactions. Crosslinking enzymes such as transglutaminases and various oxidative enzymes are used for protein crosslinking in cereal, dairy, meat and fish processing to improve product texture (Lorenzen et al. 1998; Eissa et al. 2006). The reactive groups in proteins that can crosslink enzymes include Glu, Lys, Tyr and Cys residues. Initial non-enzymatic protein denaturation is commonly employed before enzymatic crosslinking in order to increase protein crosslinking efficiency. Furthermore, new protein crosslinking may occur over food processing or storage, as severe processing and storage conditions possibly alter the existing crosslinks and generate new crosslinks, e.g. some plant-originated enzymes such as peroxidase, lipoxygenase and

catechol oxidase may cause uncontrolled modification of food proteins (Matheis and Whitaker 1987).

Enzymatic reactions are widely found in food production such as cheese making and, as described earlier in this review, enzymatic hydrolysis or crosslinking modification of proteins. *Transglutaminases* (TGs) catalyse the formation of a covalent bond between a γ -carboxyamine side chain of a peptide or protein and a free amine group including the ϵ -amino group of lysine or protein lysyl residues, causing polymerization or amine incorporation (Griffin et al. 2002). TGs can crosslink myofibrillar proteins (Sun and Arntfield 2011), gelatin (Broderick et al. 2004), milk proteins (Bönisch et al. 2007), egg yolk and white (Lim et al. 1998), fish proteins (Uresti et al. 2004), and soy and cereal proteins (Kurth and Rogers 1984; Babiker 2000). Ice cream treated with TG was found smoother and easier to scoop (Okada et al. 1993). TG is an industrially used crosslinking enzyme for meat and fish processing to improve the strength of restructured meat (Serrano et al. 2003) and aid restructuring of side-stream fish cuts and low-value non-fish origin trimmings to produce novel restructured fish products (Uresti et al. 2004). *Tyrosinase* are bifunctional enzymes and type-3 copper proteins (Lerch et al. 1986) that catalyse hydroxylation of monophenols and subsequent oxidation of diphenols to *o*-quinones (Robb 1984) which can further crosslink with lysyl, tyrosyl, histidyl and cysteinyl side chains in proteins (Selinheimo et al. 2008). *Laccases* occur as monomeric proteins and act as multi-copper enzymes catalysing oxidation of various phenolics, e.g. diphenols and polyphenols via a single-electron removal mechanism generating free radicals (Thurston 1994). Proteins are generally poor substrates for laccases and require small molecule mediators to support crosslinking (Selinheimo et al. 2008). *Sulfhydryl oxidases* catalyse the reactions for disulphide bond formation from the free thiol groups of small molecules like glutathione or to the cysteine in a polypeptide and also exhibit crosslinking activity, e.g. in dairy products (Swaigood 1977) and wheat products (Kaufman and Fennema 1987).

The combined use of different processing treatments may present advantages in improving protein functionality over single-processing treatments. For example, limited proteolysis combined with high-pressure homogenisation increased the storage modulus and postponed the onset of glycinin gelation, compared to limited proteolysis only (Luo et al. 2010). Acid treatment along with pepsin proteolysis and high-pressure homogenization could improve more effectively the emulsifying and foaming properties and the freezing–thawing resistance of soybean protein isolate, compared to acid treatment only (Yuan et al. 2012). Chen et al. (2011) reported that the combination of ultrasound pretreatment and papain hydrolysis was more effective than papain hydrolysis to modify the functionality of globular proteins such as improving the emulsifying capability of soy protein isolates and the accessibility of soy protein subunits by papain. The vast difference in the

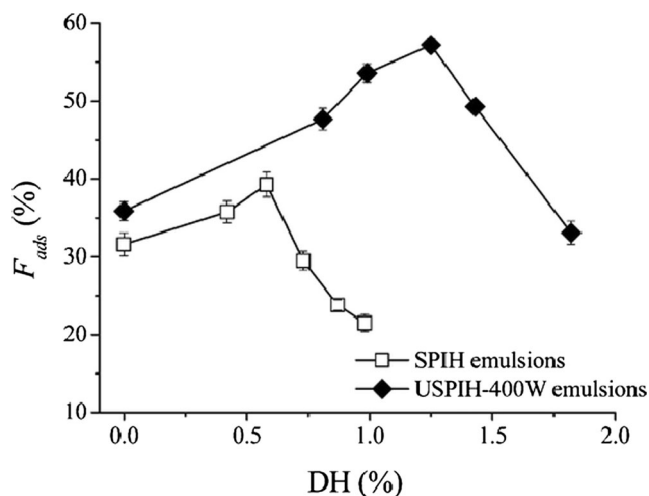


Fig. 7 Influences of degree of hydrolysis on the protein adsorption fraction (F_{ads}) of SPIH (soy protein isolate hydrolysates) and USPIH-400 W (ultrasound pretreated SPIH) during homogenization (20 % v/v oil, 1.6 % w/v emulsifier and pH=7.0) (Chen et al. 2011)

protein adsorption fraction (F_{ads} , %) that refers to the fraction of protein adsorbed onto the droplets during homogenization, reflects the different interfacial properties between the soy protein isolate hydrolysates (SPIH) and the ultrasound pretreated SPIH (USPIH-400 W) emulsions (Fig. 7). So far, only a few studies have been carried out on the combined use of different processing technologies to modify proteins, and these studies have not directly correlated the use of the combinations of processing methods with protein digestibility and food nutritional value for human.

Conclusions

Modifying proteins to gain insights into structure–function relationships has been studied extensively over the pasting decades but largely was carried out from the chemistry perspective and in laboratory settings, and has led to contradictory findings in terms of obtained functional properties. Knowledge gaps exist in terms of modifying protein and optimizing food formulation to maximally improve food processability, increase protein digestibility and retain phytochemical activity. Research on modification of protein ingredients in various food matrices during food processing, handling, ingestion and digestion remain very limited. Thus, modification of proteins to produce high nutritional quality protein ingredients and protein-rich processed foods containing incorporated bioactives (especially phytochemicals and dietary fibres) has still yet to be commercially widespread. The impact of food formulation and food processing on the functionality of modified protein ingredients cannot be excluded. Understanding protein digestion is a prerequisite for predicting the effects of processing on protein anabolism. Multi-nutritional evaluation on modified protein ingredients

in terms of protein quality and safety, nutrition labelling and potential of impaired nutrition, toxicity and allergenic response is a must to satisfy food legislation including those launched by US Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA).

Protein-rich foods especially those enhanced with bioactives attract increasing attention due to their relevance to complete and well-balanced nutrition and food shortage worldwide. Numerous reports have been published in the field of protein structure, physicochemical and nutritional properties of proteins and also their molecular interactions with other food components (including phytochemicals), although mostly from the chemistry perspective. Food protein modification has been primarily applied in fundamental studies of food chemistry or food physics. Most studies of protein-related interactions in the past were in diluted solutions or simplified protein systems. This provides basic information at molecular level, but fails in mapping with the real physiological events related to protein digestibility and bioavailability that occur in vivo or in concentrated protein-rich diets or processed foods. Food microstructure greatly influences protein digestion and bioactive release. There is a need to revisit protein sources and utilization potential as well as protein-related interactions within the new context of bioactive-enhanced protein-rich foods (i.e. functional foods) especially those containing bioactive dietary fibres and phytochemicals (which potentially impart anti-nutritional effects). Such insights are possible with the availability of new processing technologies and characterization techniques such as confocal microscopy, atomic force microscopy, surface-enhanced raman spectroscopy, light scattering, diffusive wave spectroscopy, liquid chromatography–mass spectrometry, fourier transform–mass spectrometry, solid-/solution-state NMR, fourier-transform NMR and ultrasonics as well as the evolved in vitro and in vivo digestion models. Environmentally sustainable plant-based proteins, and their rationalized combinations or blends with animal proteins, present many possible “food solutions” to consumers with different and specific needs as well as metabolic differences. Increasing utilization of plant proteins provides real and alternative approaches to address global food supply and security challenges. Improvement of transition from animal proteins to plant proteins can only be realized through satisfying all or most of the following constraints, from crop choice, use of byproducts, consequences for other natural resources, food processing and human consumption.

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