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Trends in infant formulas: a dairy perspective

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Abstract: Infant food is basically specialty nutrition with highly-balanced composition aimed at mimicking breast milk, the golden standard, as closely as possible. The baby food industry nowadays offers a wide range of products trying to fulfill the changing needs of newborns and young children in their early stages of life.

Cows' milk is the starting material of most infant food products and differs in many aspects from human milk. Humanization of infant formula therefore receives great attention. Adapting the casein-whey protein ratio and increasing the α -lactalbumin content (reducing the β -lactoglobulin content) are amongst the most obvious changes that need to be made in the final formula composition. Allergy to cows' milk is one of the possible negative effects of infant formula that can be overcome by applying hypoallergenic bovine protein hydrolysate, for which new technologies are developed to optimize taste and allergenicity.

It is realized that minor components such as oligosaccharides, nucleotides, proteins and peptides have an important function in mothers' milk and therefore technologies are developed to obtain biologically active ingredients such as bioactive peptides. Processing technologies, used to ensure the highest microbial quality of infant food, have great impact on the individual ingredients. Therefore new processing technologies such as ultra high temperature ultra short-time heating technologies and high-pressure technologies are developed and evaluated. This chapter deals with various aspects of developments of new ingredients and technologies to improve infant food.

Key words: human milk, humanization, casein, whey, minor components, processing, hydrolysates, allergenicity, peptides.

17.1 Introduction

Balanced nutrition provides the foundation for health. The first year of life is the most important period regarding growth, with many physical, psychological and mental changes taking place. Amongst others, digestive and immunological functions develop and nutritional needs change as a consequence. Infant food composition is adapted accordingly, resulting in a wide pallet of specifically tailored infant formula available nowadays for successive stages in life.

Infant food producers work continuously together with medical experts, nutritionists and international (non-governmental) bodies to increase the scientific knowledge of the special nutritional needs of infants and young children. Constant technological advances have enabled manufacturers to produce ever safer and healthier products to give children the best start in life (International Association of Infant Food Manufacturers, www.ifm.net).

Human breast milk is the best source of infant nutrition. The WHO recommends breastfeeding for, at least, the first 6 months of life. Infants who are deprived of this natural source for whatsoever reasons have infant formula as the only legitimate option. Human milk is the absolute gold standard for any infant formula, but establishing its exact composition is a very complex and still unfinished task. Based on scientific discoveries, many improvements have been realized since the origin of the first artificial infant milk based on cow's milk, including adapted whey/casein ratio, replacement of milk fats, and fortification with vitamins and minerals.

Over the last years, the focus seems to be shifting towards mimicking the functions of mother's milk rather than its exact composition. In Europe it is possible to add new ingredients to infant formula if their suitability for particular use has been well established by generally accepted scientific data (Koletzko *et al.*, 2002). New directions are, amongst others, reinforcement of the immune system, allergy, brain and eye development, as well as the improvement of digestibility. Targeting these needs will drive research into the discovery of suitable ingredients and technologies.

Starting with a brief introduction into the composition of human milk, with emphasis on its minor components, this chapter will address the current status of technological developments aimed at improving infant formula functionality, both from a nutritional as well as from a stability and safety point of view. Finally developments to produce hypoallergenic milk protein hydrolysates and bioactive peptide mixtures will be discussed.

17.2 Human milk

17.2.1 Introduction

Differences in nutrient composition of infant foods may have major effects on growth and development of neonates and, therefore, human milk acts as the gold standard for the industry. Although the complete mechanistic details remain to be identified, there is a consensus that during the postnatal period human milk,

amongst others, stimulates the development of the chemical and physical intestinal barrier functions, modulates the immune system and provides passive protection against pathogens. With bovine milk as the major source for infant food development, traditionally the focus for innovations was on major components. However, novel technologies (both analytical and processing technologies) have opened up novel opportunities for the industry in which important bioactivities from bovine milk are enriched or isolated to augment formulae functionality. In this respect, bioactive peptides as present in hydrolysates and/or as formed during gastrointestinal digestion show potential, as even nutritionally insignificant amounts may exert physiological effects (Meisel and FitzGerald, 2000) as further discussed below.

17.2.2 Human milk proteome

Novel sensitive proteomic technology developed throughout the last decade has enabled the identification of minor proteins in human milk. Especially after depletion of the major proteins secretory IgA, lactoferrin, α -lactalbumin and HSA (Palmer *et al.*, 2006) or purification of the milk fat globule membrane proteins (Fortunato *et al.*, 2003), it was possible to obtain a more comprehensive view of the proteins secreted in human milk.

17.2.3 Milk fat globule membrane proteins

Milk fat globule membrane protein (MFGMP) constitutes a fraction of milk proteins that may display important protective effects in early infancy. Recent proteomic studies have revealed in detail the composition of the MFGMP (reviewed in Cavaletto *et al.*, 2008). Summarized, to date important bioactivities that are associated with MFGMP include antiviral, antimicrobial and immunostimulating effects (Table 17.1). Since, generally, MFGMP is absent from infant formulae, supplementing formulae with MFGMP may be beneficial. This may simply be done by including milk fat which is generally not used in infant food formulation; however, relatively pure fractions for possible supplementation can be obtained by commercially relevant processing nowadays. It should be realized that specific bovine MFGMP components (like lactadherin) display less bioactivity than their human analogues (Kvistgaard *et al.*, 2004).

17.2.4 Nucleotides

Nucleotides are low-molecular-weight compounds comprising a nitrogenous base, a sugar moiety and one to three phosphate groups and belonging to the non-protein nitrogen fraction of milk. Nucleotides can be synthesized endogenously; however, they may become essential nutrients during conditions of rapid growth, malnutrition or infections. They are present in human milk as free monomeric nucleotides and nucleosides (the nucleotide metabolite form, which is the preferred form for absorption in the intestine), polymeric nucleotides

Table 17.1 MFGMPs and their postulated bioactivity

MFGMP	Activity	References
Mucins: Muc-1 Muc-X	Prevention of pathogen adhesion to the gut wall; inhibition of S-fimbriated <i>Escherichia coli</i> (causing sepsis and meningitis in newborns)	Patton, (2001), Peterson <i>et al.</i> (2001)
Lactadherin	Inhibition of rotavirus infection	Kvistgaard <i>et al.</i> (2004)
Milk fat globule epidermal growth factor-VIII (MFG-E8)	Opsonization of apoptotic cells for phagocytosis (stimulates rapid clearing of possible inflammatory cellular components)	Akakura <i>et al.</i> (2004)
Xanthine oxidoreductase	Generation of reactive oxygen species	Martin <i>et al.</i> (2004)

(DNA and RNA), and as part of a conjugate with other relevant compounds. In addition, metabolic precursors of nucleotides and nucleosides can be present. In their various forms nucleotides have a profound role in physiology as intermediates in energy metabolism, glycoconjugate synthesis and signal transduction. Supplementation studies with total potentially available nucleoside levels tuned to the specifications of human milk have demonstrated positive effects of ribonucleotide-supplemented infant formulae (reviewed in Gutierrez-Castrellon *et al.*, 2007). Effects include a better antibody response to common paediatric vaccinations and fewer episodes of diarrhoea. Importantly, no major risk for upper respiratory infections was identified, demonstrating that ribonucleotide supplementation has positive effects on infant health without any serious risk. A recent interesting observation is that the levels of the ribonucleotides 5'AMP, 5'GMP, 5'CMP and 5'IMP in human milk follow a circadian rhythm (Sanchez *et al.*, 2009). The rise of 5'AMP, 5'GMP and 5'UMP levels in human milk during the night is therefore suggested to be involved in a sleep-inducing effect of human milk.

17.2.5 Oligosaccharides

The microbiota of breast-fed infants is known to provide anti-infective properties and is a crucial factor for the postnatal development of the infant immune system. This is a general effect that cannot simply be correlated to a single factor, although it is commonly accepted that human milk oligosaccharides play a central role in this matter. The broad consensus is that these oligosaccharides in human milk display prebiotic effects since, to a large extent, these molecules are not digested by infants (German *et al.*, 2008). The neutral fraction of the human milk oligosaccharides appears as the most relevant factor for the development of the microbiota composition typically associated with breast-fed infants (bifidogenic effect), whereas the acidic fraction might play a role in the

prevention of adhesion of pathogens (Boehm and Stahl, 2007). Moreover, recently evidence was presented that human milk oligosaccharides modulate intestinal cell proliferation, differentiation and apoptosis via specialized signalling mechanisms, thus indicating that human milk oligosaccharides play a profound role in neonatal intestinal development (reviewed in Donovan, 2008).

17.2.6 Growth factors

Historically, growth factors have been identified to stimulate cell growth both *in vitro* and *in vivo* (hence the name growth factors). However, nowadays it is realized that these molecules have diverse functions important for the healthy development of the neonate. Importantly, of the growth factors present in human milk many exceed concentrations as present in maternal plasma, suggesting that human milk has a potent growth factor activity (Grosvenor *et al.*, 1993). Biological activities of growth factors from milk include, among others, the stimulation of growth and maturation of the gastrointestinal tract, recovery after gastrointestinal injury, control of serum insulin, and immunological protection of the GI mucosal surface (Donovan and Odle, 1994). Importantly, synergistic effects with other human milk components may occur, indicating that formula supplementation *per se* might not always be fully effective.

17.2.7 Immunoglobulins

Immunoglobulins are not transferred across the placenta to the mammalian foetus and hence infants are born with very low concentrations of serum immunoglobulins. Immunoglobulins occur in high concentration in human colostrum to provide immunological protection to the mammary gland. In addition, the absorption of immunoglobulins by infants provides passive immunity after birth, since the antibodies protect the newborn against infections (Wheeler *et al.*, 2007). The major immunoglobulins in human milk are IgA, IgG1, IgG2 and IgM. IgG1 is the main immunoglobulin type in colostrum, whereas IgM, IgA and IgG2 are present at lower concentrations. Colostrum contains approximately 100-fold higher concentrations of immunoglobulins than bovine milk and total immunoglobulin concentration decreases progressively postpartum (Marnila and Korhonen, 2002). Both IgG and IgM antibodies have multiple functions including opsonization (i.e. accentuating pathogens) to enhance phagocytosis, complement fixation for pathogen lysis, prevention of adhesion of pathogens to tissues, inhibition of microbial metabolism by blocking enzymes, agglutination of bacteria and neutralization of toxins and viruses. In contrast, secretory IgA is only involved in processes relevant to the gastrointestinal tract, such as agglutination of bacteria, prevention of microbial adhesion to the epithelial wall and neutralization of toxins and viruses. Thus, dietary immunoglobulins may provide additional protection to the newborn.

17.2.8 Non-protein nitrogen

Compared with the milk of other species, human milk is unique in the sense that non-protein nitrogen (NPN) constitutes 20–25% of the total nitrogen. NPN components include nucleotides and their metabolites nucleosides (discussed above) (Ferreira, 2003), urea (Goedhart and Bindels, 1994), amino sugars (including sialic acid) (Nakano *et al.*, 2001), free amino acids, uric acid (Ferreira, 2003), orotic acid (Ferreira, 2003), ammonia, creatine (and creatinine) (Hülsemann *et al.*, 1987), polyamines (mainly spermine, spermidine and putrescine) (Loser, 2000), growth factors (discussed above) and amino alcohols (choline and ethanolamine) (Schuel *et al.*, 2002).

17.3 Humanization of infant food

17.3.1 Introduction

A basic formulation of infant food consists of a protein fraction, a carbohydrate fraction (lactose, starch, maltodextrin, sucrose) as energy source, and a source of fatty acids, such as vegetable oil, vitamins and minerals. As stated above, the composition of human milk clearly differs from that of other mammals such as cows, which is mostly applied as a basis for infant foods, although the milks of other mammals such as donkey, goat and mare are also known to be applied as ingredients in infant foods. Humanization of infant foods, that is the adaptation of the infant food formulation towards the composition of human milk, especially as far as its protein part is concerned, has been facilitated largely by innovations in separation technologies in the dairy industry, since the end of the last century.

17.3.2 Casein–whey protein ratio and minor components

Usage of bovine whey was first facilitated by innovations in filtration technology resulting in desalted whey preparations that enabled the formulation of products having a more human-like casein–whey protein ratio. Not only does the ratio of casein to whey protein differ from that of bovine milk (40:60 human versus 82:18 bovine), human milk also differs in lacking β -lactoglobulin, the major whey protein in bovine milk. Human milk shares this characteristic with camel milk (Kappeler *et al.*, 2003). Technologies aimed at obtaining α -lactalbumin-enriched protein preparations were developed based on differences in heat-induced aggregation behaviour of the major whey proteins, α -lactalbumin and β -lactoglobulin (Pearce, 1983). Especially in acidic conditions, the calcium-free form of α -lactalbumin becomes more susceptible to heat-induced aggregation. A second, upcoming and still developing, technology to produce these kinds of preparations is (ion-exchange) chromatography. Already for years this technique has been used to produce α -lactalbumin-enriched protein preparations. This technology is currently also applied to isolate lactoferrin and lactoperoxidase, two minor components in bovine whey, applicable to

humanize infant foods, as human milk is much richer in lactoferrin compared to bovine milk (Lonnerdal, 2009). It is expected that these techniques will be further developed to obtain preparations of other minor components, such as hormones and growth factors, from bovine milks or other sources (Park and Haenlein, 2006) for further fine-tuning of the compositional mimicking of human milk.

17.3.3 Processing and (bio)functionality

Even if the mimicking of the composition of human milk could be completed to the level of minor components, another large difference between infant foods and human milk would still exist. In contrast to human milk, infant food is processed and stored. On the other hand, during their production and storage infant foods are exposed to conditions that may affect the nutritional and functional properties of the ingredients. For example, to ensure the microbial quality of the final product, heating is applied. A major side-effect of this heating is that protein denaturation and subsequent aggregation occur (Tanford, 1968). In addition other biologically active components, such as vitamins and growth factors, may be destroyed as well. Moreover, protein modifications can occur, such as Maillard-type reactions between sugars and proteins (glycosylation) resulting in covalently linked sugar–protein complexes. In milk, the protein fraction can react with the lactose present (so-called lactosylation). An example of the effect of processing on the lactosylation of proteins in bovine milk is shown in Fig. 17.1. The observed broadening of the peaks in the reversed-phase HPLC protein separation profile is mainly caused by lactosylation of the milk proteins. It can be clearly seen that the two different types of heat treatments result in different degrees of peak broadening. This means that different degrees of lactosylation are reached during these treatments. As glycosylation of proteins may affect digestibility and bioavailability of proteins, the original

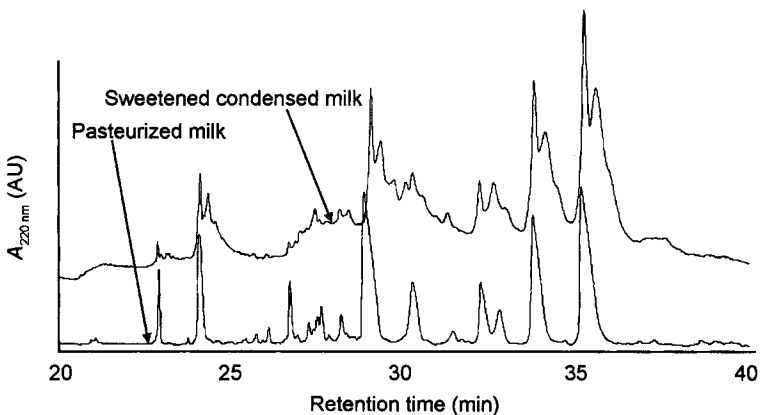


Fig. 17.1 Comparison of RP-HPLC protein separation profiles present in differently processed milk products.

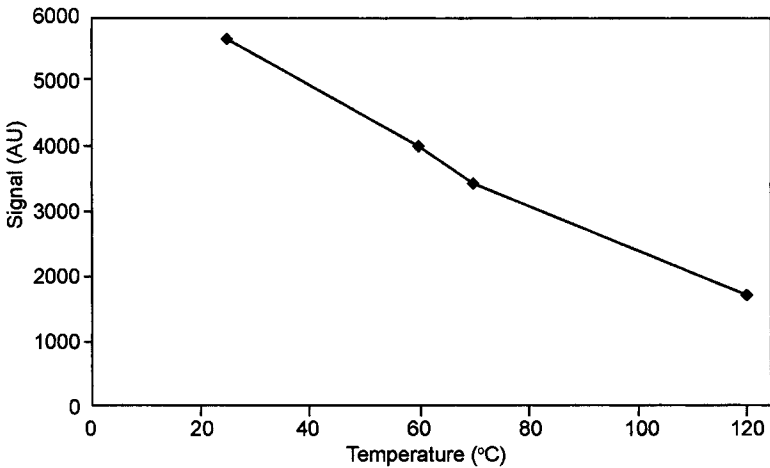


Fig. 17.2 GlcNAc1 (N-acetyl- δ -glucosamine) profile of freshly isolated cheese-whey treated at different temperatures, mimicking industrial processing (heat-loads).

functionality of these milk proteins will most likely be changed as a consequence of the processing.

In addition to protein aggregation and lactosylation, heating could also affect endogenous glycosylation of proteins, such as the complex glycosylation of α -lactalbumin, lactoferrin and κ -casein. Figure 17.2 shows the correlation between applied heating of bovine whey and the detected levels of GlcNAc1 (N-acetyl-D-glucosamine). These kinds of complex glycosylated components are thought to play an important physiological role and their biological activity is at least in part related to the sugar moieties (Dziuba and Minkiewicz, 1996).

In conclusion, during processing of infant foods and especially during the applied heating steps, reactions will occur that affect the integrity of ingredients. As a result it may be expected that these modifications will affect the functionality of the ingredients. For example, it is generally known that lactosylation will decrease the number of available lysine residues and may affect digestibility, bioavailability and possibly allergenicity. It is expected that denaturation/aggregation reactions will affect the biological function of the modified component, since this biological activity is mostly related to the conformation of the native molecules (e.g. lactoferrin and lactoperoxidase are inactive when denatured).

17.3.4 New preserving technologies, innovative steam injection

In order to assure the safety of infant foods, micro-organisms have to be inactivated. This means that processing steps will always be required. The most commonly applied technique to achieve this is heat treatment. As described, however, the drawback is that heating may impair the quality of foods, causing unacceptable loss of functionality, such as destruction of vitamins, protein

denaturation and formation of off-flavours. An additional problem, which has emerged in recent years, is spoilage of foods by very heat-resistant bacterial spores originating from raw materials. So-called minimal processing techniques like high-pressure treatment and pulsed electric fields (PEF) appear to be insufficient to inactivate bacterial spores (De Jong and Heesch, 1998; Pol *et al.*, 2001). The application of ultra-high temperatures ($>150^{\circ}\text{C}$) and ultra-short residence times ($<0.2\text{ s}$) might offer new opportunities with regard to microbial inactivation while preserving product quality (Van Asselt *et al.*, 2008). This new time–temperature profile allows significant inactivation of heat-resistant spores (46 decimal reductions), while preserving the (bio)functionality of important ingredients to a large extent.

17.3.5 Enhancing function versus mimicking composition

Processing mostly affects functionality of ingredients as stated above. Therefore, it may be questioned if ‘complete’ humanization of infant foods can be reached by completely mimicking the composition of human milk. Infant foods will always differ from human milk in the degree of processing and this will affect the functionality of the ingredients in infant foods. Therefore, a practical approach is to aim for equivalent functionality rather than for an equal composition. The focus here is to retain or add a specific biological functionality. A recent example is the application of a mixture of galacto/fructo-oligosaccharides (GOS/FOS) to modulate the infants’ intestinal microflora towards one that is rich in bifidobacteria as observed in breast-fed infants (Bakker-Zierikzee *et al.*, 2005). This bifidogenic effect of human milk has been recognized for many years, and naturally present complex (branched) oligosaccharides have been recognized as the responsible components (Boehm *et al.*, 2007).

GOS is another innovative example of how the dairy industry develops ingredients from whey. GOS is an oligosaccharide resulting from the enzymatic action of β -galactosidase on lactose. FOS is obtained from chicory extract. Since the majority of oligosaccharides from bovine milk have a different composition from those present in human milk and levels may be too low to provide for a sufficient biological effect (Gopal and Gill, 2000; Tao *et al.*, 2008), other ingredients that mimic the functionality of human milk oligosaccharides can be applied.

Another very illustrative example is the addition of probiotic bacteria to infant formula. Rather than stimulating beneficial gut bacteria through oligosaccharide supplementation, this concept deals with the actual addition of beneficial health-promoting bacteria. Probiotics have benefits in steering the immune response as has recently been demonstrated (van Baarlen *et al.*, 2009). Until recently it was thought that human milk did not contain bacteria, except for carry-over from the skin, and therefore the addition of bacteria would fall within this category of mimicking function rather than composition. However, recent findings may warrant a review of this statement (Lara-Villoslada *et al.*, 2007).

17.4 Towards optimized composition: analytical tools and models

With human milk as a reference, development of functional infant formulae requires sensitive analytical methodologies. Nowadays several specialized techniques aiming at target identification, quantification of bioactives and quality control are available, enabling continuous innovation in infant food. For example, methods for quantification of lactoferrin, oligosaccharide profiling (Ninonuevo *et al.*, 2006), comparative and quantitative proteomics (Goshe and Smith, 2003), phospholipid and nucleotide quantification have been designed.

17.5 Infant food and allergenicity

17.5.1 General introduction

For the production of infant foods, bovine milk has been the major source. Above it was discussed that although, to the largest extent, the composition of human milk can be mimicked, differences will always exist because of processing-induced changes in (bio)functionality. In addition, it has been realized for many decades that cow's milk allergy (CMA) is a major issue in the production of infant formula. Allergy, best described as an inappropriate response against innocuous antigens, is, in most cases, caused by proteins. Allergy to cow's milk proteins is the most common allergy in infancy. Cow's milk is usually the first food antigen introduced in an infant's diet, and therefore cow's milk allergy is often the first presentation of the atopic constitution of an individual (Sampson, 2004). Although CMA is often transient in childhood, children with CMA are at greater risk of developing other atopic disorders, including atopic dermatitis, allergic asthma and allergic rhinitis. Although tolerance to cow's milk develops in more than 85% of infants before the age of 3 years (Bock, 1987; Host and Halken, 1990; Jakobsson and Lindberg, 1979), a small number retain their allergy. Cow's milk allergy in adults is rare but usually severe. Recently it was shown that in these patients both caseins and whey proteins play a negative role (Lam *et al.*, 2008). In fact the major infant formulae producers have been focusing on reduction of allergenicity almost from the start of their business.

In order to be successful in the development of these formulae, it is vital to know what exactly is causing cow's milk allergy. The protein fraction of milk consists of caseins (α_{s1} -, α_{s2} -, β - and κ -casein) and whey proteins (α -lactalbumin and β -lactoglobulin being the most abundant). The IgE response during allergy is generally directed to all major proteins (van Beresteijn *et al.*, 1995) as shown in Fig. 17.3.

The casein fraction of cow's milk protein appears to be more allergenic than whey proteins in both children and adults (Stoger and Wuthrich, 1993; Szabo and Eigenmann, 2000) and from the individual caseins α_{s1} -casein was shown to be the most potent allergen and κ -casein the least allergenic (Bernard *et al.*, 1998).

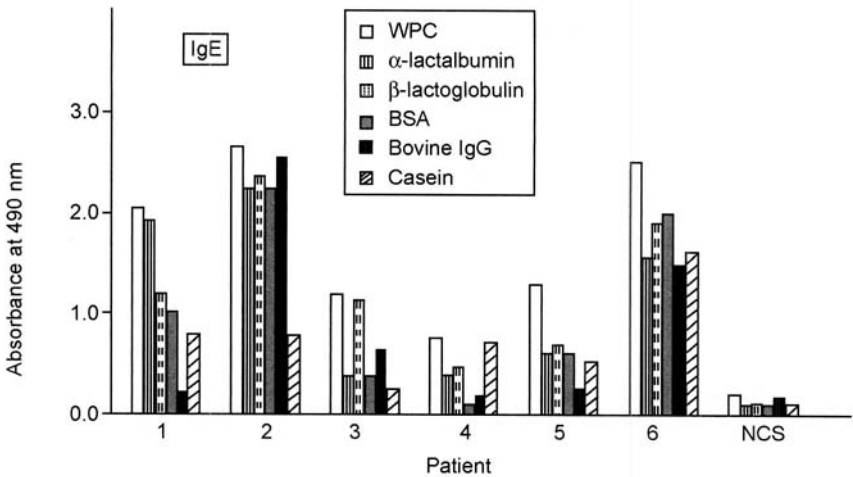


Fig. 17.3 Serum levels of specific IgE antibodies in six patients against casein, bovine α -lactalbumin, bovine β -lactoglobulin, BSA and bovine immunoglobulins.

On the other hand, β -lactoglobulin, the major whey protein, is also claimed to be one of the major milk allergens (Wal, 2004). This protein is absent from human milk and relatively resistant to proteolytic attack and gastric acid. Moreover, during the first months in life this protein is able to cross the intestinal barrier intact in infants.

Under normal conditions, after digestion in the stomach, peptides and amino acids enter the duodenum, where further degradation takes place before absorption via the mucosal membrane, following which they serve as nutrients. Oligopeptides below eight amino acids (smaller than 3000 Da) are not presented or recognized by immune cells and are therefore immunologically inert (Suri *et al.*, 2006). However, a small part of food proteins are not fully degraded and can elicit immune responses after absorption (Untersmayr and Jensen-Jarolim, 2006). Especially in young children, due to the immaturity of the digestive system (and the higher pH in the stomach), the amount of partly degraded proteins absorbed will be higher (Lonnerdal, 2003). This increases the chance of the presence of immunologically active milk protein fragments that can be recognized as foreign by the immune system, resulting in the induction of immune responses. The best infant nutrition is human milk, because in addition to having many factors (e.g. immunoglobulins, lysozyme, lactoferrin, lactoperoxidase, growth factors) that provide passive immunity and protection to the newborn, its protein is not allergenic (Lonnerdal, 2003).

17.5.2 Technologies to produce hypoallergenic infant formula

The infant food industry has developed various methods and processing technologies to reduce allergenicity of cow's milk proteins, which are generally used in infant formula. Enzymatic hydrolysis of bovine milk proteins, aimed at

eliminating allergenic epitopes, is the most effective strategy that has led to successful hypoallergenic infant food products (Host and Halken, 2004). The optimal hydrolysis with respect to immunological properties and nutritional value is, however, unknown and strongly depends on the type of proteins present in the starting material (e.g. whey or casein-based). The main challenge, when using hydrolysis, is maintaining palatability (bitterness is the most prevalent problem here), nutritional properties and functional properties of the original proteins (Host and Halken, 2004).

That enzymatic hydrolysis of cow's milk protein in infant formulas is an effective strategy to reduce overall allergenicity was, amongst others, demonstrated by a study from van Beresteijn *et al.*, in which the serum of patients allergic to cow's milk was shown to have antibodies to fractions of cow's milk and the residual antigenicity of hydrolysates was lower than that of intact proteins (van Beresteijn *et al.*, 1995). However, immunoreactive epitopes could still be detected in all tested, commercially available products. Even a 3000 Da hydrolysate was shown to contain IgE epitopes, although this particular hydrolysate did not elicit allergic reactions in experimental animals (van Beresteijn *et al.*, 1994) suggesting that, in this case, physiological digestion reactions could completely eliminate residual epitopes.

A combination of selective hydrolysis (using a lactococcal extracellular envelope protease) of caseins in a milk protein mixture followed by ultra-filtration (cutoff values between 3 and 30 kDa) resulted in casein hydrolysates free of protein fragments that were cross-reactive with whey-protein-specific IgE (Alting *et al.*, 1998). In addition, casein itself was shown to be degraded in such a way that cross-reactive casein-specific IgE antibody-binding sites were eliminated, showing that the epitopes in caseins were completely destroyed. This hydrolysate out-performed commercially available hydrolysates in terms of hypoallergenicity.

Thus, for hydrolysis aiming at allergy reduction the choice of the enzyme or enzyme combinations is very important, even if only low molecular weight fractions are taken as protein material with reduced allergenicity (Wroblewska *et al.*, 2007). For example, pepsin, the protease that naturally occurs in the stomach, was shown to be hardly effective in reducing the antigenicity of cow's milk protein hydrolysates in combination with other proteases.

The goal for these hydrolysis processes is always to eliminate the epitopes of the milk proteins. The exact nature of these epitopes, however, is unknown, which hampers rational approaches. At least for β -lactoglobulin, a systematic approach was used to get more insight into the parts of the molecule that were recognized by T-cells from patients with cow's milk allergy, and seven peptides were identified to which the T-cells from these patients responded, producing high levels of Il-5 (Inoue *et al.*, 2001).

In conclusion, milk protein hydrolysis is an effective strategy to produce hypoallergenic formula; however, the production of an effective hydrolysate may still be further optimized: specific destruction of epitopes is still an important target.

17.5.3 High pressure

A new approach to eliminate allergenic milk protein epitopes more efficiently is the use of physical treatments to denature the proteins before enzymatic treatment, thereby providing better substrates for the proteases, expected to result in more efficient hydrolysis (Bonomi *et al.*, 2000). High pressure is a rather new technology that nowadays is finding its way into industrial practice, although production-scale application remains a challenge. Under certain temperature and high pressure conditions β -lactoglobulin forms transient conformations with regions of the hydrophobic core unfolded. These new protein conformations present new target peptide bonds for enzymatic hydrolysis, as compared with the native protein (Bonomi *et al.*, 2003).

For β -lactoglobulin it was shown that high-pressure treatment in the presence of the enzymes trypsin and chymotrypsin indeed leads to a reduction of IgG binding (Chicón *et al.*, 2008). This is in line with the finding that pressure-induced changes of globular whey proteins have an effect on the proteolysis (Belloque *et al.*, 2007). However, peptides containing IgE epitopes were still formed under the conditions used. It was therefore suggested that screening for the right conditions (and enzyme combinations) would lead to the development of hydrolysates with a further reduced allergenicity. Indeed such an approach was followed and was shown to be successful, i.e. screening of different enzymes under various conditions led to the development of hypoallergenic bovine whey protein hydrolysates (Peñas *et al.*, 2006). This shows the potential of combining physical technologies, such as high hydrostatic pressure, and enzyme technologies for further development of hypoallergenic milk protein hydrolysates for the infant food industry. This can be of great interest for the industry, since in commercially available hypoallergenic milk formulas potentially allergenic material can still be detected, such as residual antigenic β -lactoglobulin, even in extensively hydrolysed products (Rosendal and Barkholt, 2000).

17.6 Other beneficial properties of milk protein hydrolysates

17.6.1 Increased rate of uptake

Besides reduction of formula allergenicity, milk protein hydrolysates are also used in infant food to improve overall digestibility of the formula and provide a better nutrient uptake. In fact, it has been shown that the uptake of amino acids in the form of peptides (di- and tri-peptides) occurs more readily than that of free amino acids in man (Silk *et al.*, 1982). This is another important reason to use hydrolysed milk proteins in infant formulas.

17.6.2 Bioactive peptides

In recent years it has been recognized that milk proteins are a rich source of biologically active peptides. These peptides, encrypted in the milk protein, are

inactive within the sequence of the parent protein, but can be released by enzymatic hydrolysis. Upon ingestion these peptides may affect major physiological systems, namely the digestive, immune and nervous systems, depending on their amino acid sequences. These peptides may also act locally, e.g. in the gut to modulate the intestinal microbiota. Especially since particular peptides are potent and may exert physiological effects in nutritionally insignificant amounts, dietary peptides have a large potential to promote health effects (Korhonen and Pihlanto, 2006). An overview is given in Table 17.2 of specific groups of bioactive peptides that can be derived from milk proteins.

The antimicrobial peptides are of particular interest, since these peptides may play a role in maintaining a healthy gut microflora. In this respect lactoferricin (a potent antimicrobial lactoferrin-derived peptide) has been detected along the gastro-intestinal tract of mice (Kuwata *et al.*, 1998), indicating the potential *in vivo* activity. In order to obtain efficacy data to support a new or improved infant formula *in vivo* nutritional intervention trials are essential. There is a need to reduce the number of these (animal) trials in discovery programmes for ethical and financial reasons. Up to now predictive computer models aiming to circumvent and/or reduce the number of these *in vivo* experiments are rare or too complex, with many non-measurable adjustable parameters. Recently a basic physico-chemical computer model for a first quantitative interpretation of results obtained from *in vivo* intestinal experiments with bacteria was developed (de Jong *et al.*, 2007). This new modelling approach was validated with results obtained from gut infection studies *in vivo* and could be used to test the hypothesis that the growth rate of *E. coli* in the colon of the rat and man is different.

There is a large number of milk-derived peptides that could display antibacterial or antiviral properties (Floris *et al.*, 2003) and this potential of milk

Table 17.2 Overview of bioactive peptides and their potential effects that can be derived from the various milk proteins by means of enzymatic hydrolysis

Protein source	Effect	Name
α -, β -caseins	Mineral binding	Phosphopeptides
κ -casein	Opioid antagonist	Casoxin
α -, β -, κ -caseins	Opioid agonist	Casomorphins
α -, β -, κ -caseins	Immune modulation	Immunopeptides
α -, κ -caseins	Antimicrobial	Isracidin/casocidin-I/ casecidin
κ -casein	Antithrombotic	Casoplatelins
α -, β -caseins	Antihypertensive	Casokinins
α -lactalbumin	Opioid agonist, ACE inhibition	α -lactorphin
β -lactoglobulin	Non-opioid stimulatory effect on ileum, ACE inhibition	β -lactorphin
	Ileum contraction	β -lactotensin
BSA	Opioid activity	Serorphin
	Ileum contraction, ACE inhibition	Albutensin A
Lactoferrin	Bactericidal activity	Lactoferricin

protein hydrolysates to display biological effects is not yet exploited by the infant food industry. Therefore peptides offer large opportunities for further innovations and development of new products. In these product development trajectories the challenge will be to balance the hypoallergenic properties of a hydrolysate with the presence of the desired bioactive peptides. Importantly, since many hydrolysates are already utilized in infant foods, establishing the presence of possible physiological active peptides in these hydrolysates may be a first step in identifying novel functionalities in available infant formula.

17.6.3 Tolerogenic peptides

Finally, peptides can play a role in inducing oral tolerance to milk proteins, defined as the specific suppression of cellular and/or humoral immune response to an antigen by prior administration of the antigen via the oral route (Faria and Weiner, 2005). If oral tolerance fails, food allergy occurs. In animal models, oral tolerance against food proteins has been achieved with the use of moderately hydrolysed proteins as inducers. Induction of such a specific oral tolerance by tolerogenic peptides (peptides that induce tolerance) derived from food proteins is seen as a powerful measure to drag the immune system towards tolerance instead of sensitization. For β -lactoglobulin it has now been shown that specific peptides derived from this protein indeed could be used to make mice tolerant against β -lactoglobulin. Tolerogenic peptides could be a very important tool for the infant food industry to help overcome milk protein allergy in children.

17.6.4 Reduction of bitterness

Bitterness is a major drawback of hydrolysed food products and especially infant food formulae. Caseins are notoriously bitter when hydrolysed (Lemieux and Simard, 1992) and therefore casein-derived hydrolysates have not been applied widely in the food industry unless intensively hydrolysed. This bitter off-taste represents a technical problem that has not been adequately solved by the food industry (Pawlett and Bruce, 1996). There are some general masking approaches to reduce bitterness, such as addition of artificial sweeteners, addition of flavours, addition of bitter blockers (e.g. adenosine monophosphate) and processing measures (e.g. ultrafiltration, column chromatography) to remove bitter peptides. However, none of these approaches reduces bitterness sufficiently, and moreover in many cases, due to strict regulation, these measures are not always allowed in infant food preparation.

Recently, however, a novel approach was followed using a proline-specific enzyme that resulted in debittering of casein hydrolysates (Edens *et al.*, 2005). Such new approaches are very promising and could lead to the development of a new generation of hydrolysates for the infant food industry by combining low allergenicity with reduced bitterness.

17.6.5 Use of other protein sources: rice proteins

A widely used approach to help children with cow's milk allergy is to switch to hydrolysates based on other proteins. The most common approach here is to prescribe soy formula. However, allergic reactions to soy formulas are known amongst atopic children (Bruno *et al.*, 1997). It was therefore investigated whether rice hydrolysate formula could be used for children with cow's milk and soy allergy. Indeed, children with these allergies could tolerate such a hydrolysate, indicating that rice protein is a promising food source for children with multiple food-induced allergies (Fiocchi *et al.*, 2003). In common practice this novel source, however, needs to be further evaluated.

17.7 Conclusions

Human milk is the best source of nutrition for infants. Many attempts have been and are being undertaken in order to fully unravel its composition and functionalities. This is largely driving the development and improvement of infant formula by industry (reviewed by Nasirpour *et al.*, 2006), resulting in foods with specific nutritional properties targeted at specific age categories and/or need states. One of today's hottest scientific interests is the understanding of the impact of early infant feeding on the metabolic state in later life, especially in relation to (childhood) obesity. As a result of such increasing scientific understanding, the incorporation of speciality ingredients (not rarely non-dairy) such as pre- and probiotics, and long chain, polyunsaturated fatty acids (mainly derived from fish oil), known to be important in visual and brain development amongst others, has been and will be driving infant formula innovations. Protecting these usually sensitive components during processing, storage and food preparation is a major challenge and requires the continuous optimization of technologies such as micro-encapsulation and mild (heat) processing.

Of major future importance will be the allergy epidemic that is foreseen. Two major ingredient classes play an important role. The need for carefully selected and designed protein hydrolysates will continue to increase, not only guaranteeing hypo-allergenicity, but comprising additional bioactive functionalities because of carefully designed peptide patterns. Secondly, pre- and probiotics have been identified as a means to affect immune priming. Clinical trials are being published, but true scientific mechanistic understanding is lacking. Scientific research in this area will greatly expand in the coming years and will eventually show the potential of these ingredients.

Industry cannot restrict its efforts to these nutritional innovations, since infant foods require usually a long shelf-life, which demands significant processing to ensure food safety. This has a very serious impact not only on their nutritional quality but also their physical stability, and the organoleptic properties are affected, which creates the need for innovative technological solutions. Typically the control of sensoric properties of protein hydrolysates as well as off-flavour management will be ongoing areas of attention. A rather

undeveloped area would be the understanding of the behaviour of foods in the infantile digestive tract. As an example, textural and flavour properties influence food liking and intake, as well as stomach emptying and satiety. In times of heavy competition and demanding consumers, addressing these latter aspects could make the additional difference.

17.8 References

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