World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:4, No:12, 2010

Bioactive Component in Milk and Dairy Product

Bahareh Hajirostamloo

Abstract**—**Recent research has shown that milk proteins can yield bioactive peptides with opioid, mineral binding, cytomodulatory, antihypertensive, immunostimulating, antimicrobial and antioxidative activity in the human body. Bioactive peptides are encrypted in milk proteins and are only released by enzymatic hydrolysis in vivo during gastrointestinal digestion, food processing or by microbial enzymes in fermented products. At present significant research is being undertaken on the health effects of bioactive peptides. A variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt, sour milk and cheese. In particular, antihypertensive peptides have been identified in fermented milks, whey and ripened cheese. Some of these peptides have been commercialized in the form of fermented milks. Bioactive peptides have the potential to be used in the formulation of health-enhancing nutraceuticals, and as potent drugs with well defined pharmacological effects.

Keywords**—**Milk protein, Bioactive peptides, Health effects, Dairy product.

I. INTRODUCTION

MANY food proteins can exert a physiological action, either directly or, after their degradation, in the form of fragments. Peptides represent a quite heterogeneous class of compounds and their characteristics deeply depend on the amino acidic composition and on the length of the chain. The acid-basic behavior is determined by the free terminal residues and by the ionic lateral group of the residues in the chain; the reactivity of the terminal groups is also useful for their detection and quantification. Protein physic-chemical properties remarkably change after degradation and, consequently, some oligopeptides may play an important role in determining the rheological characteristics of a food. In fact they have been successfully used as additives as long as they are more soluble, less viscous and with greater emulsifying and foaming properties than the native proteins [1]. Peptides are generally tasteless or bitter except for dipeptides containing Glutamic or Aspartic acid which, typically sweet, are widely used in food industry since they do not cause dental caries and do not contribute to obesity or pathologies such as diabetes mellite. Bitter peptides, such as dichetopiperazins and cyclic dipeptides, have been found in caseinic hydrolysates [2], and in cheeses [3]. The bitter taste is mainly correlated to the hydrophobic amino acid content [4] and to the increasing length of the chain, though over a certain length, it is no more perceptible. Nevertheless, an extended hydrolysis of the proteins may produce peptides with a very intense, bitter taste [5].

Bahareh Hajirostamloo, Young Researcher Club, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran, phone: 00989141614155, (E-mail: Hajirostamlo_m@yahoo.com).

In food matrices containing sugars, some peptides can undergo the Maillard reaction by heating process, thus modifying the appearance of the product; in milk, lactose and lysine residues in proteins (mainly in caseins) can give rise to a series of undesirable brown pigments and aromatic compounds which weigh upon the color and the flavor of heated milk [6].

From a nutritional point of view, peptides represent a more bio available form of essential amino acids than proteins, even compared to free amino acids, both in terms of increasing assimilation rate at the brush border membrane of the human intestine and of reducing osmotic pressure [7]. In this context, protein hydrolysates and peptides with definite characteristics are being used for clinical applications: for instance, peptide preparations, rich in branched-chain amino acids and poor in aromatic amino acids, have been related to improved conditions in patients affected by liver encephalopathy and they could be addressed to potential nutrition and to people suffering from hepatic pathologies [8].

Low molecular weight peptides are also less allergenic than native proteins; therefore, milk protein hydrolysates are commonly utilized to formulate hypoallergenic food for infants [9].

The physiological activity of some peptides, able to positively affect human health, has attracted the interest of researchers and the food industries.

II. BIO-ACTIVE PEPTIDES

Bioactive peptides are described as food-derived components (genuine or generated) that, in addition to their nutritional value, exert a physiological effect in the body [10]. In 1950, Mellander first described bioactive peptides when he reported that ingestion of casein-derived phosphorylated peptides led to enhanced vitamin D-independent calcification in rachitic infants. Since then, fundamental studies have opened a new field of research related to the generation of bioactive peptides from a variety of food proteins, with milk proteins currently being the primary source. Bioactive peptides can be latent (or encrypted) within the primary or parent proteins, where proteolysis is required for their release and activation to exert a physiological response [11] on the various systems in the body. Some peptides also act as biocarriers by sequestering calcium and other minerals, thereby enhancing bioavailability [12].

III. SOURCE OF BIOACTIVE PEPTIDES

Milk contains a wide range of proteins that provide protection against enteropathogens or are essential for the

manufacture and characteristic nature of certain dairy products [17]. Milk has been shown to contain an array of bioactivities, which extend the range of influence of mother over young beyond nutrition [11]. Peptides are in a latent or inactive state within protein molecules but can be released during enzymatic digestion. Biologically active peptides released from caseins and whey proteins contain 3 to 20 amino acids per molecule [17]. Researchers for the last decade have demonstrated that these bioactive peptides possess very important biological functionalities, including antimicrobial, antihypertensive, antioxidative, anticytotoxic, immunomodulatory, opioid, and mineral-carrying activities.

Most of the bioactivities of milk proteins are latent, being absent or incomplete in the original native protein, but full activities are manifested upon proteolytic digestion to release and activate encrypted bioactive peptides from the original protein [13], [14]. Bioactive peptides (BPs) have been identified within the amino acid sequences of native milk proteins. They may be released by proteolysis during gastrointestinal transit or during food processing. Enzymes such as digestive, naturally occurring in milk, coagulants and microbial enzymes, especially those from adventitious or lactic acid starter bacteria usually generate these bioactive compounds. BPs are released from milk proteins during milk fermentation and cheese maturation, which enriches the dairy products [11].

IV. Production of Bioactive Peptides

Bioactive peptides are inactive within the sequence of the parent protein and can be released in three ways: (a) enzymatic hydrolysis by digestive enzymes, (b) food processing and (c) proteolysis by enzymes derived from microorganisms or plants.

A. Enzymatic hydrolysis

The cleavage of latent bioactive peptides from milk proteins normally occurs during digestion by pepsin and pancreatic enzymes (trypsin, chymotrypsin, carboxy and aminopeptidases), producing active peptide fragments in the gastrointestinal tract of the milk-consuming individual [15] The physiological effects of bioactive peptides depend on their ability to reach their target sites intact, which may involve absorption through the intestinal epithelium prior to travel to the peripheral organs [10].

Many of the known bioactive peptides have been produced *in vitro* using gastrointestinal enzymes, usually pepsin and trypsin. ACE-inhibitory peptides and CPPs, for example, are most commonly produced by trypsin. Other digestive enzymes and different enzyme combinations of proteinases -including alcalase, chymotrypsin, pancreatin, pepsin and thermolysin as well as enzymes from bacterial and fungal sources- have also been utilized to generate bioactive peptides from various proteins.

B. Food Processing

The structural and chemical changes that occur during the processing of food proteins may result in the release of bioactive peptides. In particular, heat and/or alkali treatment can generate additional inter-and intramolecular covalent bonds that are resistant to hydrolysis. Such processing conditions also promote the racemic conversion of L-amino acids to D-isomers and consequently, lead to indigestible peptide bonds. The potential formation of indigestible peptide sequences during food processing is noteworthy, because this may promote both formation and absorption of bioactive peptides that do not occur naturally in the precursor protein. Such bioactive peptides can be generated during manufacture of several milk products and may thus be ingested as food components. For example, partially hydrolyzed milk proteins for hypoallergenic infant formulae and for clinical applications in nutrition consist exclusively of peptides and contain bioactive peptides. Cheese contains phosphopeptides as natural constituents and secondary proteolysis during cheese ripening leads to formation of various ACE inhibitory peptides.

C. Microbial fermentation

Many industrially utilized dairy starter cultures are proteolytic to some extent. Bioactive peptides can, thus, be generated by the proteolytic activities of the strains of starter and non-starter bacteria e.g. *Lactobacillus helveticus, Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus, Lactococcus lactis, Streptococcus thermophilus* used in the manufacture of fermented dairy products. The proteolytic system of lactic acid bacteria (LAB) is well characterized. This system consists of a cell wall-bound proteinase and a number of distinct intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases and dipeptidases. Extracellular proteinases cause degradation of casein into oligopeptides. The longer chain oligopeptides may be a source of bioactive peptides when further degraded by intracellular peptidases of lysed-lactic acid bacteria.

The single most effective way to increase the concentration of bioactive peptides in fermented dairy products is to ferment or co-ferment with highly proteolytic strains of LAB. The choice of strains influences the release of effective bioactive peptides. The strain should not be too proteolytic otherwise the product will be destroyed and must have the right specificity to give high concentrations of active peptides. The concentration of ACE-inhibitory peptides seems to rely on a balance between their formation and further breakdown into inactive peptides and amino acids that in turn depends on storage time and conditions. Various bioactive peptides including ACE-inhibitory or antihypertensive peptides, immunomodulatory, antioxidative, antimutagenic peptides have been released from milk proteins through microbial proteolysis [11], [16], [17], [15]. The best known ACE-inhibitory peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP), have been identified in milk fermented with strains of *Lb. helveticus* and *Saccharomyces cerevisiae.* In addition to live microorganisms, proteolytic enzymes isolated from LAB have

been successfully employed to release bioactive peptides from milk proteins.

V. Physiological Effects of Bioactive Peptides

A. Effects on cardiovascular system

Hypertension is one of the major risk factors for cardiovascular disease and stroke. Because diet has a role in the prevention and treatment of this disease, there is interest in developing foods with antihypertensive activity. ACE is a multifunctional ectoenzyme that is located in many tissues and plays an important role in blood pressure regulation and in turn hypertension. Therefore, ACE inhibition mainly results in a hypotensive effect. Recent research has shown that enzymatic digestion of casein and whey proteins generate peptides that have the ability to inhibit ACE. The best known ACE-inhibitory peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) with IC 50 values (concentration of peptides mediating 50% inhibition of ACE activity) of 9 and 5 μMoles respectively have been identified from a Japanese sour milk drink (Calpis ®) fermented with *Lb. helveticus* and *Saccharomyces cerevisiae* strains [19]. In a placebo-controlled study, the blood pressure of hypertensive patients decreased significantly after 4 weeks of daily ingestion of 95 ml of sour milk that contained these two tripeptides. This was equivalent to an ingested dose of ACE inhibitory peptides of about 2.6 mg per day [20]. Similarly, a milk product Evolus ® fermented with *Lb. helveticus LBK-16H* (Valio Ltd, Finland or Kaiku Vitabrand ®, Spain) exerted significant antihypertensive effects in humans at daily doses of 150 ml. Two other commercial products, a casein hydrolysate containing the peptide FFVAPFPEVFGK (α_{s1}-casein f23-34; Casein DP, Kanebo, Ltd, Japan, and C12 peptide, DMV, The Netherlands) and a whey protein hydrolysate (BioZate, Davisco, US) were also claimed to lower blood pressure in humans [21].

B. Effects on immune system

Diet is known to play an important role in the body's defense mechanism. Research concerning the role of functional peptides on the immune system is quite recent but seems to be promising. The two main activities being studied are the immunomodulatory (stimulation of immune system) and antimicrobial (inhibition of microorganisms) effects of bioactive peptides. Several casein and whey protein derived peptides display an immunomodulatory role. Immunomodulating peptides have been found to stimulate the proliferation of human lymphocytes, the phagocytic activities of macrophages and antibody synthesis. Also, it has been suggested that immunomodulatory milk peptides may alleviate allergic reactions in humans and enhance mucosal immunity in the gastrointestinal tract [22]. In this way immunomodulatory peptides may regulate the development of the immune system in newborn infants. Furthermore, it has been suggested that immunopeptides formed during milk fermentation may contribute to the antitumor effects of fermented milk [18].

The antimicrobial properties of milk have been widely acknowledged for many years. The antimicrobial activity of milk is mainly attributed to immunoglobulins, and to non-immune proteins, such as lactoferrin, lactoperoxidase and lysozyme. One of the most potent antimicrobial peptides described so far corresponds to a fragment of the whey protein lactoferrin, named lactoferricin [23]. More recently, other whey proteins such as α-lactalbumin and *ß*-lactoglobulin have also been considered as potential precursors of bactericidal fragments. Similarly, antibacterial fragments have also been derived from α_{s1}-, α_{s2}- and κ-casein [24], [25]. These peptides have been found to be active against a broad range of pathogenic organisms e.g. *Escherichia, Helicobacter, Listeria, Salmonella* and *Staphylococcus,* yeasts and filamentous fungi. Depending on the target microorganism, inhibitory concentrations of peptides vary, eg: antimicrobial peptides α_{s2} -CN f183-207 and f164-179 exhibited inhibition against Gram-positive and -negative bacteria with minimal inhibitory concentrations (MICs) ranges from 8 to 95 μmol/l [25].

C. Effects on nervous system

Recent studies have provided evidence that peptides exist in dairy products which play an active role in the nervous system; these are known as opioid peptides. The first major opioid peptides discovered were *ß*-casomorphins, fragments of *ß*-casein [26]. Once absorbed into blood, these peptides can travel to the brain and various other organs and elicit pharmacological properties similar to opium or morphine. This may be the reason why human neonates generally become calm and sleepy after drinking milk. In contrast to the casomorphins, some peptides produced by the break down of κ-casein function as opioid antagonists, that is, they can inhibit the effect of morphine like substances.

D. Effects on nutritional status and dental health

Casein-derived phosphorylated peptides, CPPs, can form soluble organophosphate salts and lead to enhanced mineral uptake. Calcium absorption is enhanced by limiting the precipitation of calcium in the distal ileum. The charged side chains, in particular the phosphate groups of amino acids can bind minerals e.g. Ca, Mg, Fe and Zn [27]. Since CPPs can bind and solubilise minerals, they may have value in the prevention of osteoporosis, dental caries, hypertension and anemia. CPPs can have an anticariogenic effect by promoting recalcification of tooth enamel, whereas glycomacropeptide (GMP) derived from κ-casein seems to contribute to the anticaries effect by inhibiting the adhesion and growth of plaque-forming bacteria on oral mucosa. Various dental care products containing CPPs and/or GMP are now commercially available.

E. Other functional roles

Recent studies have shown that peptides with antioxidative properties can be released from caseins by hydrolysis with digestive enzymes and by proteolytic LAB in fermented milks [22]. Most these were derived from α_s-casein and have been

shown to possess free radical-scavenging activities and to inhibit enzymatic and non-enzymatic lipid peroxidation. In the future, antioxidative peptides may find applications as ingredients in different fields, e.g. in the prevention of oxidation in fat-containing foodstuffs, cosmetics and pharmaceuticals. More research is needed to demonstrate if peptides produced during fermentation can prevent oxidative damage *in vivo.* Nagaoka *et al.* [28] identified a hypocholesterolemic peptide (Ile-Ile-Ala-Glu-Lys) from the tryptic hydrolysate of ß-lactoglobulin. This peptide suppressed cholesterol absorption by Caco-2 cells *in vitro* and elicited hypocholesterolemic activity *in vivo* in rats upon oral administration of the peptide solution. The mechanism of the hypocholesterolemic effect remains to be clarified.

VI. Future perspectives for bioactive peptides

The potential health benefits of milk protein-derived peptides have been a subject of growing commercial interest in the context of health-promoting functional foods. So far, antihypertensive, mineral-binding and anti-cariogenic peptides have been most studied for their physiological effects. A few commercial developments have been launched on the market and this trend is likely to continue alongside with increasing knowledge about the functionalities of the peptides. The optimal exploitation of bioactive peptides for human nutrition and health possesses an exciting scientific and technological challenge, while at the same time offering potential for commercially successful applications. Bioactive peptides can be incorporated in the form of ingredients in functional and novel foods, dietary supplements and even pharmaceuticals with the purpose of delivering specific health benefits. Such tailored dietary formulations are currently being developed worldwide to optimize health through nutrition. This approach has been taken initially at target group level but will ultimately address individuals. Bioactive peptides offer an excellent basis for the novel concept of ‘‘personalized nutrition’’. Many scientific, technological and regulatory issues must, however, be resolved before these substances can be optimally harnessed to this end.

Firstly, there is need to develop novel technologies, such as chromatographic and membrane separation techniques, to enrich active peptide fractions from the hydrolysates of various food proteins [29], [30], [31], [32]. In addition to enzymatic hydrolysis, microbial fermentation provides a natural technology applicable for the production of bioactive peptides either from animal or plant proteins. The potential of this approach is already well demonstrated by the presence of bioactive peptides in fermented dairy products. Production of bioactive peptides from protein-rich raw materials may be scaled up to industrial level using control led fermentation in bioreactors with known LAB. In the future, the commercial production of specific peptide sequences is likely to employer combined enzyme technology and specific production strains or alternatively makes use of peptidases isolated from suitable microorganisms.

Secondly, it is important to study the technological properties of active peptide fractions and to develop model foods that contain these peptides and retain their activity for a guaranteed period. It is recognized that, due to their lower molecular weight, peptides can be more reactive than proteins, and the peptides present in the food matrix may react with other food components. The interaction of peptides with carbohydrates and lipids, as well as the influence of the processing conditions (particularly heating) on peptide activity and bioavailability, should also be addressed [33]. In this respect, the possible formation of toxic, allergenic or carcinogenic substances warrants intensive research. Modern analytical methods need to be developed to study the safety of food stuffs containing biologically active peptides.

Thirdly, molecular studies are needed to assess the mechanisms by which bioactive peptides exert their activities. For this approach, it is necessary to employ proteomics and associated technologies [34]. By developing such novel facilities it will be possible to study the impact of proteins and peptides on the expression of genes and hence optimize the nutritional and health effects of these compounds. This research area is currently considered highly challenging and will revolutionize the protein research in the near future. The majority of the known bioactive peptides are not absorbed from the gastrointestinal tract in to the blood circulation and their effect is, therefore, probably mediated directly in the gut lumen or through receptors on the intestinal cell wall. In this respect, the target function of the concerned peptide is of utmost importance. It is anticipated that such targets will be related to various lifestyle-related disease groups, such as cardiovascular diseases, cancers, diabetes, osteoporosis, stress and obesity. Bioactive pep-tides derived from milk proteins offer a promising approach for the promotion of health by means of a tailored diet and provide interesting opportunities to the dairy industry for expansion of its field of operation.

References

[1] F. Tidona, A. Criscione, A.M. Guastella, A. Zuccaro, S. Bordonaro and D. Marletta, “Bioactive peptides in dairy products”, *Ital. J. Anim. Sci.*, vol. 8, pp. 315-340, 2009.

[2] E. Minagawa, S. Kaminogawa, F. Tsukasaki and K. Yamauchi, “Deittering mechanism in bitter peptides of enzymatic hydrolysates from milk casein by aminopeptidase”, *T. J. Food Sci.*, vol. 54, pp. 1225-1229, 1989.

[3] K.D. Lee and J.J. Warthesen, “Preparative Methods of Isolating Bitter Peptides from Cheddar Cheese”, *J. Agricult. Food Chem.*, vol. 44, pp. 1058-1063, 1995.

[4] A.J. Cliffe and B.A. Law, “Peptide composition of enzyme-treated Cheddar cheese slurries determined by reverse phase high performance liquid chromatography”, *Food Chem*., vol. 36, pp. 73-80, 1990.

[5] G.E. Vegarud and T. Langsrud, “The level of bitterness and solubility of hydrolysates produced by controlled proteolysis of caseins”, *J. Dairy Res.*, vol. 56, pp. 375-379, 1989.

[6] M.A. Matin, M. Monnai and H. Otani, “Isolation and characterization of a cytotoxic pentapeptide □ -casecidin, from bovine □ -casein digested with bovine trypsin”, *J. Animal Sci.*, vol. 71, pp. 197-207, 2000.

[7] S.A. Adibi, “The oligopeptide transporter (PEPT1) in human intestine: biology and function”, *Gastroenterology*, vol. 113, pp. 332-340, 1997.

[8] S. Adachi, Y. Kimura, K. Murakami, R. Matsuno and H. Yokogoshi, “Separation of peptide groups with definite characteristics from enzymatic protein hydrolysate”, *Agric. Biol. Chem*., vol. 55, pp. 925-

932, 1991.
[9] A. Host and S. Halken, "Hypoallergenic formulas - when, to whom and how long: after more then 15 years we know the right indication", *Allergy*, vol. 59, pp. 45-52, 2004.
[10] V. Vermeirssen, J. Van Camp and W. Verstraete, "Bioavailability of angiotensin I converting enzyme inhibitory peptides", *British Journal of Nutrition*, vol. 92, pp. 357–366, 2004.
[11] M. Gobetti, F. Minervini and C.G. Rizzello, "Angiotensin I-converting-enzyme-inhibitory and antimicrobial bioactive peptides", *International Journal of Dairy Technology*, vol. 57, pp. 173–188, 2004.
[12] S.V. Silva and F.X. Malcata, "Caseins as source of bioactive peptides", *International Dairy Journal*, vol. 15, pp. 1-5, 2005.
[13] D.A. Clare and H.E. Swaisgood, "Bioactive milk peptides: A prospectus", *Journal of Dairy Science*, vol. 83, pp. 1187-1195, 2000.
[14] M. Gobbetti, L. Stepaniak, M. De Angelis, A. Corsetti and R. Di Cagno, "Latent Bioactive Peptides in Milk Proteins: Proteolytic Activation and Signiicance in Dairy Processing", *Crit. Rev. Food Sci. Nutr*., vol. 42, pp. 223-239, 2002.
[15] E. Schlimme and H. Meisel, "Bioactive peptides derived from milk proteins. Structural, physiological and analytical aspects", *Die NMahrung*, vol. 39, pp. 1-29, 1995.
[16] H. Korhonen and A. Pihlanto, "Food-derived bioactive peptides—opportunities for designing future foods", *Current Pharmaceutical Design*, vol. 9, pp. 1297–1308, 2001.
[17] H. Korhonen and A. Pihlanto, A., "Milk-derived bioactive peptides: formation and prospects for health promotion" in *Handbook of functional dairy products. Functional foods and nutraceuticals,* C. Shortt and J. O'Brien Eds. CRC Press: Boca Raton, Florida, USA, 2004, pp. 109-124.
[18] C. Matar, J.G. LeBlanc, L. Martin and G. Perdigón, "Biologically active peptides released in fermented milk: Role and functions", in *Handbook of fermented functional foods. Functional foods and nutraceuticals,* E.R. Farnworth, Ed. CRC Press: Boca Raton, Florida, USA, 2003, pp. 177–201.
[19] Y. Nakamura, N. Yamamoto, K. Sakai and T. Takano, "Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme", *Journal of Dairy Science*, vol. 78, pp. 1253–1257, 1995.
[20] I. Hata, J. Ueda and H. Otani, "Immuno stimulatory action of a commercially available casein phosphopeptide preparation CPP-III, in cell cultures", *Milchwissenschaft*, vol. 54, pp. 3-7, 1999.
[21] R.J. Fitzgerald, B.A Murray and D.J. Walsh, "Hypotensive peptides from milk proteins", *Journal of Nutrition*, vol. 134, pp. 980S–988S, 2004.
[22] H. Korhonen and A. Pihlanto, "Food-derived bioactive peptides—opportunities for designing future foods", *Curr. Pharm. Design*, vol. 9, pp. 1297-1308, 2003.
[23] W. Bellamy, M. Takase, K. Yamauchi, H. Wakabayashi, K. Kawase and M. Tomita, "Identification of the bactericidal domain of lactoferrin", *Biochimica et Biophysica Acta*, vol. 1121, pp. 130–136, 1992.
[24] E. Lahov and W. Regelson, "Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides", *Food Chemical Toxicology*, vol. 34, pp. 131–145, 1996.
[25] I. Recio and S. Visser, "Two ion-exchange methods for the isolation of antibacterial peptides from lactoferrin—in situ enzymatic hydrolysis on an ion-exchange membrane", *Journal of Chromatography*, vol. 831, pp. 191–201, 1999.
[26] E. Smacchi and M. Gobbetti, "Peptides from several Italian cheeses inhibitory to proteolytic enzymes of lactic acid bacteria, Pseudomonas fluorescens ATCC 948 and to the angiotensin I-converting enzyme", *Enzyme and microbial technology*, vol. 22, pp. 687–694, 1998.
[27] H. Meisel, "Overview on milk protein-derived peptides", *International Dairy Journal*, vol. 8, pp. 363–373, 1998.
[28] S. Nagaoka, Y. Futamura, K. Miwa, T. Awano, K. Yamauchi and Y. Kanamaru, "Identification of novel hypocholesterolemic peptides derived from bovine milk β-lactoglobulin", *Biochemical and Biophysical Research Communications*, vol. 218, pp. 11–17, 2001.
[29] H. Korhonen, "Technology options for new nutritional concepts", *International Journal of Dairy Technology*, vol. 55, pp. 79–88, 2002.
[30] R. Mehra, and P.M. Kelly, "Whey protein fraction at ion using cascade membrane filtration", *IDF Bulletin*, vol. 389, pp. 40–44, 2004.
[31] A. Tolkach and U. Kulozik, "Fractionation of whey proteins and peptides by means of membrane techniques in connection with chemical and physical pretreatments", *IDF Bulletin*, vol. 389, vol. 20–23, 2004.
[32] K. DeSilva, R. Stockmann and G.W. Smithers, "Isolation procedures for functional dairy components-novel approaches to meeting the challenges", *Australian Journal of Dairy Technology*, vol. 58, pp. 148–152, 2003.
[33] H. Korhonen, A. Pihlanto-Leppala, P. Rantamaki and T. Tupasela, "Impact of processing on bioactive proteins and peptides", *Trends in Food Science and Technology*, vol. 9, pp. 307–319, 1998.
[34] R. O'Donnell, J.W. Holland, H.C. Deeth and P. Alewood, "Milk proteomics", *International Dairy Journal*, vol. 14, pp. 1013–1023, 2004.