Biofunctional properties of milk protein derived bioactive peptides - A review

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ABSTRACT

Milk has long been acknowledged as a source of macro- and micro nutrients. Presently, several identified biologically active substances from milk and their derivatives has attracted much attention from the scientific community. These bioactive compounds confer many health benefits that might support disease prevention. Worldwide, there is an increasing interest in the therapeutic potential of bioactive peptides which collectively present a cornucopia of activities for utilization in humans. Bioactive peptides are hydrolysates with specific amino acid sequences that exert a positive physiological effect on the body. Most of the biological activities are encrypted within the primary sequence of the native protein and can be released during digestion by proteolytic enzymes in the gastrointestinal tract or during fermentation and food processing. Milk protein is an important source of bioactive peptides which may contribute to regulate the nervous, gastrointestinal, and cardiovascular systems as well as the immune system. Milk protein derived bioactive peptides are shown to have antihypertensive, antimicrobial, immunomodulatory, antioxidative and mineral-binding properties. Bioactive peptides derived from milk proteins are of particular interest to the food industry due to the potential functional and physiological roles that they exhibit.

Key words: Bioactive peptides, Fermentation, Food industry, Milk, Milk protein.

An increasing amount of scientific evidence confirms that many chronic diseases such as cancer, osteoporosis, coronary heart diseases, and hypertension are connected to an unbalanced diet (Everitt et al., 2006). Among the main food stuffs, milk has been identified to be of particular interest with regard to ailment and health. It is the exclusive source of nutrients for the young and it also represents a high-grade source of dietary nitrogen and indispensable amino acids for adults. Recently, the role of protein in the diet as a physiological active component is being increasingly acknowledged worldwide. Milk protein, an important source of amino acid is very well accepted, but in current times, it has been recognized that milk proteins show numerous functionalities in vivo by the action of bioactive peptides (Ricci-Cabello et al., 2012). The protein in our daily diet varies from 95 to 120 g, which is hydrolysed into a large variety of peptides during gastrointestinal digestion. Some of these peptides show structural uniqueness with endogenous peptides that act in the organism as hormones, neurotransmitters, or regulatory peptides. These exogenous food-derived peptides can interact with the same receptors in the organism and exert an agonistic or antagonistic activity. There are many examples of food-derived peptides structurally similar to endogenous peptides; one of the most representative examples is opioid peptides, which have been demonstrated to behave as opioid receptors ligands (Teschemacher, 2003).

Presently, milk proteins are considered as the most vital resource of a range of bioactive peptides (Ricci-Cabello et al., 2012). Bioactive peptides are specific protein fragments that have a positive impact on the functions or conditions and may ultimately influence health (Kitts and Weiler, 2003). Fermented dairy products and other food containing bioactive peptides would appear to have the potential to offer specific health benefits to consumers. Milk protein derived active biological constituents, bioactive peptides, has received much attention for the biological significances, and is currently the subject of intensive research. Once liberated and absorbed these bioactive peptides may exert a physiological effect on the various systems of the body, for example the cardiovascular, digestive, endocrine, immune and nervous system (Hayes et al., 2006). Milk peptides have been shown to possess multiple physiological properties, including antimicrobial activity, opioid activity, anti-hypertension activity, modulation of digestive enzymes, nutrient absorption, and immune responses (Fig 1). Because of their physiological and physicochemical versatility, milk peptides are regarded as very important constituents for incorporation in functional and novel foods, dietary supplements, and even pharmaceuticals with the purpose of targeting specific disease.

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On the other hand, to exert a biological function in an organism, there is no requirement of absorption of peptides. The gut is considered to be the largest endocrine organ in the body with a large range of endogenous peptides secreted and receptors expressed. The gastrointestinal tract is in contact with food digested and therefore, is a source of variety of food-derived peptides. For this reason, the effects of peptides on different intestinal functions and health are attracting an increasing interest (Moughan et al., 2007; Shimizu and Hachimura, 2011).

**Production of milk protein derived bioactive peptides:** Milk-derived bioactive peptides can be encrypted in both casein (α, β, and γ casein) and whey proteins (β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, and proteose-peptone fractions) and can be released from their parent proteins by following four ways (Phelan et al., 2009) (Fig. 1):

(i) Enzymatic hydrolysis during gastrointestinal digestion  
(ii) Fermentation of milk with proteolytic starter cultures  
(iii) Hydrolysis by enzymes obtained from microorganisms or plants  
(iv) Combination of fermentation and hydrolysis

If the sequence of the peptide is known, it is also possible to synthesize peptides by chemical route, recombinant DNA technology or enzymatic amide synthesis (Madureira et al., 2010). After releasing from origin, bioactive peptides must reach the target receptor in the intestinal lumen or in other peripheral organs, passing via the systematic circulation. The most commonly used enzymes are pepsin, trypsin, and chymotrypsin, of animal as well as microbial origin. In cheese and fermented milk, the production of bioactive peptides results from the synthesis of metabolites with proteolytic capability by bacteria from milk-protein substrate (Fitzgerald and Murray, 2006).

Depending on the number and/or sequence of the amino acids, food-derived bioactive peptides can display various activities by binding to a specific receptor in the gastrointestinal tract or in target organs and tissues after absorption into the bloodstream.

In recent time, there has been a growing interest in the use of dairy hydrolysates containing bioactive peptides as agents for maintaining general health and preventing chronic human diseases. Therefore, several technologies, principally based on the enzymatic hydrolysis, have been developed for the production of these bioactive hydrolysates (Phelan et al., 2009). In the food industry, the enzymatic membrane reactor allows protein hydrolysis and the subsequent separation of peptides generated by microfiltration or chromatography techniques, such as size exclusion or ion-exchange chromatography (Welderufael et al., 2012). Moreover, instead of traditional methods, subcritical water hydrolysis strategies have been proposed for dairy hydrolysates production without use of acids, bases, or enzymes. In addition, high hydrostatic pressure causes substantial modifications to milk proteins, and ultimately influences their functional properties.

**Role of milk protein derived bioactive peptides**

(i) **Effect on the cardiovascular system**

**Antihypertensive peptides:** Elevated blood pressure is one of the risk factors for cardiovascular disease. ACE (EC 3.4.15.1) plays important role in the regulation of blood pressure; thus, inhibition of this enzyme is one of the strategies for the management of hypertension. *In vitro* and *in vivo* studies found that antihypertensive peptide sequences was present in both casein and whey fractions (Contreras et al., 2012). The tryptic CN hydrolysate containing the peptide ₂S₁-CN f(23-34) has been patented and commercialised as antihypertensive peptide under the name of C12®. Another, two peptides derived from ₂S₁-CN from pepsin casein hydrolysate with sequences RYLGY and AYFYPEL, showed potent systolic blood pressure (SBP) reducing effects in animal model (Contreras et al., 2009). Besides, antihypertensive peptides are also produced in fermented milks and cheeses by the use of proteolytic system of lactic acid bacteria. For example, the production of β-CN-derived peptides, IPP and VPP, in sour milk fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae* (Calpis®) has potent SBP decreasing effects.

**Anti-inflammatory peptides:** Chronic inflammation is related to many age related diseases, such as atherosclerosis, vascular diseases, arthritis, cancer, diabetes, osteoporosis, dementia, obesity, and metabolic syndrome. Different cytokines play a pivotal role as mediators in the production of biomarkers implied in the progression of inflammation and the endothelial dysfunction. The down-regulation of cytokines which are responsible for the production of biomarkers implied in the progression of inflammation by
using peptides may retard or alleviate inflammation, hence exerting beneficial effects against cardiovascular diseases (Tompa et al., 2010). To date, only the commercial peptide NOP-47, derived from whey, enhanced the vascular function by modulating glucose levels and biomarkers of inflammation (Ballard et al., 2009).

**Antioxidant peptides:** Antioxidant peptides from milk proteins play a vital role in the maintenance of antioxidant defense systems by averting the formation of free radicals or by scavenging free radicals and active oxygen species, which induce oxidative damage to biomolecules and cause aging, cancer, heart disease, stroke, and arteriosclerosis. So far, many antioxidant peptides derived from both CN and whey proteins have been characterised (Power et al., 2013). The peptide Tyr-Phe-Tyr-Pro-Glu-Leu from a peptic digestion of casein had a strong superoxide anion radical-scavenging activity, attributed mainly to the C-terminal amino acid Glu-Leu. The β-casein f (169–176) fraction was found to have the greatest inhibitory effect on linoleic acid oxidation. Peptide derived from the β-lactoglobulin A Trp-Tyr-Ser-Leu-Ala-Met-Ala-Ala-Ser-Asp-Ile, has higher radical-scavenging activity than butylated hydroxy anisole. Some antioxidant peptides have also been isolated from fermented dairy products, such as the sequence Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met, which has been isolated from milk fermented with Lactobacillus delbrueckii ssp. bulgaricus and shows a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity (Ricci-Cabello et al., 2012).

**Hypocholesterolaemic peptides:** It is necessary to maintain appropriate ratio of blood lipids, as it is one of the most important risk factors for developing cardiovascular diseases (CVD). Milk proteins, particularly whey proteins hydrolyzates or peptides, have been reported to exert hypocholesterolaemic effects in different animal models. The ingestion of whey protein was correlated with a significant reduction of total cholesterol levels in rats fed with cholesterol-free and cholesterol-enriched diets. A similar effect was found for the β-lactoglobulin trypic hydrolysates in rats fed with diet rich in cholesterol. Protein fragment f (71–75) with sequence IIAEK, known as lactostatin, was the main factor responsible for observed effect (Nagaoka et al., 2001). Another peptide β-Lactotensin, obtained from chymotrypsin β-lactoglobulin hydrolysate, decreased total cholesterol, LDL, and VLDL cholesterol content in mice fed with a cholesterol enriched diet (Yamauchi et al., 2003).

**Antithrombotic peptides:** The peptides released from k-casein by the action of rennin, which inhibit blood platelet aggregation and fibrinogen binding (γ-chain) to platelet surface receptors are encrypted within the sequence of glycomacropeptide (Chabance et al., 1995). The peptide from k-CN f (113–116) has been isolated from yoghurt with antithrombotic activity (Dionysiou et al., 2000). An antithrombotic peptide PPK from k-CN f (109–111), has the same structure homology with an earlier identified antithrombotic peptide MAIPPK which was isolated from the water-soluble extract of Spanish fermented milk drinks (Hernandez-Ledesma et al., 2004). Fractions corresponding to κ-CN f (152–160) and f (155–160) isolated as ACE-inhibitors may also possess antithrombotic activity (Gobbetti et al., 2004).

**Effect on the gastrointestinal system**

**Mineral binding peptides:** Worldwide, mineral deficiencies are the most important nutritional problems. In this context, mineral fortification is one of the best and most common strategies to prevent this deficiency (Zimmermann and Hurrell, 2007). It has been proposed that the phosphorylated regions released from casein during digestion. CPPs refer to casein-derived phosphorylated peptides, which contain single and multiple phosphoryl residues, and these phosphopeptides are released by enzymatic hydrolysis of α, β- and κ-caseins both in vitro and in vivo (Clare and Swaisgood, 2000). Due to the high content of negative charges, these peptides efficiently bind divalent cations and act as biocarriers for trace elements such as Fe, Mn, Cu and Se. CPPs generally refer to peptides generated after enzymatic treatment with trypsin and which enhance absorption of calcium across the distal small intestine (Pfeuffer and Schrzenmeier, 2000). CPPs are used in the food industry as ingredients or fortifiers in some low mineral-containing foods and drinks.

**Antidiabetic peptides:** It was suggested that the antidiabetic properties of milk protein are primarily attributable to its content of bioactive peptides which, following their release during gastrointestinal digestion, could arouse the secretion of gut-derived hormones and/or inhibit enzymes involved in glucose homeostasis. One of the remedial strategies for managing T2D is to decrease postprandial hyperglycemia by retarding the absorption of glucose through inhibition of carbohydrate-hydrolyzing enzymes, e.g. alpha-glucosidase, in the digestive organs (Slama et al., 2006). Inhibition of this enzyme in digestive tract delays carbohydrate digestion and increases overall carbohydrate digestion time. Hence, less glucose is absorbed because the carbohydrates are not rapidly hydrolyzed down into glucose molecules and subsequently diminishing the postprandial blood glucose and insulin level. Results from one study demonstrated that peptides with inhibitory property against alpha-glucosidase activity can be generated from the peptic digestion of whey proteins (Lacroix and Li-chan, 2013).

Dipeptidyl peptidase IV (DPP-IV/CD26; EC.3.4.14.5) is a multifunctional transmembrane glycoprotein. It is a 766–amino acid serine protease that contains N-terminal dipeptidases activity which selectively cleaves dipeptides after proline or alanine residues. Gastrointestinal
hormones, including GIP and GLP-1, are endogenous substrates for the enzyme DPP-IV. The two incretin hormones, GIP and GLP-1, are secreted by enteroendocrine cells of the intestines within minutes of food ingested and increase nutrient-stimulated insulin secretion in a glucose-dependent manner (Drucker and Nauck, 2006). Thus, DPP-IV inhibitors exert their positive effect on glucose regulation by slowing down the rapid inactivation of endogenous GIP and GLP-1, thus enhancing insulin secretion. Various studies have highlighted that milk protein is the natural source of DPP-IV inhibitors (Lacroix and Li-Chan, 2012; Tulipano, et al., 2011; Uenishi, et al., 2012). Different peptides were identified for the DPP-IV inhibitory activity of the water soluble fraction of Gouda-type cheese. The β-casein peptide residue 70-77 (β-CN f70-77; LPQNIPPL) showed the highest DPP-IV inhibitor activity (Uenishi, et al., 2012).

Antioesity peptides: In regulation of food intake, satiety plays an important role and has significance in the control of obesity. It is very well accepted that protein is the most satiating component of food. The satiating effect of whey protein is mainly due to a high concentration of branch chain amino acids, particularly L-leucine. Regarding the casein fraction of milk, it was proposed that peptides from casein hydrolysates activates the peripheral opioid and cholecystokinin receptors and blocks the antagonist receptors which reduces their effect on food intake (Hernández-Ledesma et al., 2013). Several studies showed that the whey protein derived peptide i.e. glycomacropeptide (GMP) stimulates the release of cholecystokinin (CCK), which may promote satiety in rats (Pedersen et al., 2000). Some peptides derived from casein and whey proteins may stimulate the synthesis of the glucagon-like peptide-1, which suppresses the intake of food by increasing the sensation of satiety.

Antimicrobial peptides: The antimicrobial activity of milk is due to the synergistic activity of naturally occurring peptides and defense proteins besides immunoglobulins, such as lactoferrin, lactoperoxidase and lysozyme. The first antimicrobial peptide isolated from milk by the action of rennet, termed lactein, was identified by Simmes and Jones in 1930. This peptide exhibited antimicrobial activity against pathogenic strains of streptococci. The peptide called casecidins from chymosin treated casein hydrolysates exhibited antimicrobial activity against pathogenic Staphylococcus aureus and several lactobacilli. Consequently, the antimicrobial peptide termed isracidin corresponding to αs1-casein f (1-23) inhibited the growth of S. aureus and L. monocytogenes (Lahov and Regelson, 1996). An amphiphilic and a positive charge of the peptides are important characters determining the interaction with bacterial membranes. Lactoferricin is one of the most potent antimicrobial peptide from the whey protein lactoferrin and antimicrobial properties can be related to tryptophan/arginine rich proportion of the peptide (Vogel et al., 2002). The antimicrobial activity of the peptides might be due to disruption of microbial membranes, leading to ion and metabolite leakage, depolarization, disruption of membrane coupled respiration and ultimately cell death (Phadke et al., 2005).

Effect on the immune system

Immunomodulatory peptides: Milk protein hydrolysates and peptides enhance immune cell functions by lymphocyte proliferation, antibody synthesis and cytokine regulation. Several peptides were identified, namely f63-68 and f191-193 from bovine β-casein and f194-199 from bovine αs1-casein which stimulate phagocytosis in mice and humans in vitro and protect against Klebsiella pneumonia infection in mice in vivo (Tidona et al., 2009). Kayser and Meisel (1996) reported that di- and tri-peptides like Tyr-Gly and Tyr-Gly-Gly from bovine κ-casein and α-lactalbumin respectively, significantly increased the proliferation of human peripheral blood lymphocytes in vivo. It is found that CN, whey proteins, and their different hydrolysates show modulatory effects on the immune system in both in vitro and in vivo studies. Furthermore, immune peptides formed during milk fermentation have been shown to contribute to the antitumoural effects observed in many studies with fermented milks.

Cytomodulatory peptides: Cytomodulatory peptides inhibit the growth of cancer cell and stimulate the activity of immune competent cells. The peptidic fractions from milk fermented with Lb. helveticus showed cytomodulatory effects in mice (LeBlanc et al., 2002). Additionally, cytomodulatory effects in a human breast cancer cell line were reported from the peptides obtained from the milk fermented with probiotic milks. Several peptides were identified, namely f63-68 and f194-199 from bovine αs1-casein and f194-199 from bovine αs1-casein which stimulate phagocytosis in mice and humans in vitro and protect against Klebsiella pneumonia infection in mice in vivo (Tidona et al., 2009). Kayser and Meisel (1996) reported that di- and tri-peptides like Tyr-Gly and Tyr-Gly-Gly from bovine κ-casein and α-lactalbumin respectively, significantly increased the proliferation of human peripheral blood lymphocytes in vivo. It is found that CN, whey proteins, and their different hydrolysates show modulatory effects on the immune system in both in vitro and in vivo studies. Furthermore, immune peptides formed during milk fermentation have been shown to contribute to the antitumoural effects observed in many studies with fermented milks.

CONCLUSION

The potential health benefits of bioactive milk peptides have been a subject of growing commercial interest in the context of health promoting functional foods. An adequate intake of milk and milk products as a part of a healthy, balanced diet is needed throughout life to promote health. Scientific studies signify that milk contains a plethora of bioactive peptides that can positively have an impact on human health. The interest on bioactive milk peptides is
increasing because milk proteins are available in great amounts with a high degree of purity at low price, which, under a technological aspect, makes them attractive in the search of bioactive peptides. These bioactive peptides can be produced and made bioavailable through proteolysis by the action of digestive enzymes or through the fermentation by bacteria. Further work is also required to synthesize modified peptide sequences in order to find pharmacologically active peptides with a higher potency and longer duration of action. Furthermore, there is a need to develop technologies by means of which active peptide fractions can be produced and enriched.

REFERENCE


