Milk -derived Bioactive Peptides

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ABSTRACT

A variety of milk-derived biologically active peptides have been shown to exert both functional and physiological roles in vitro and in vivo, and because of this are of particular interest for food science and nutrition applications. Biological activities associated with such peptides include immunomodulatory, antibacterial, anti-hypertensive and opioid-like properties. Milk proteins are recognized as a primary source of bioactive peptides, which can be encrypted within the amino acid sequence of dairy proteins, requiring proteolysis for release and activation. Fermentation of milk proteins using the proteolytic systems of lactic acid bacteria is an attractive approach for generation of functional foods enriched in bioactive peptides given the low cost and positive nutritional image associated with fermented milk drinks and yoghurt. Bioactive peptides have been isolated from many protein sources such as soy proteins, gelatine, fish proteins; and maize, but milk proteins appear to be the most important sources of bioactive peptides identified thus far. Milk is an excellent source of highly valuable proteins which are in general divided into caseins and whey proteins. Caseins and whey proteins comprise approximately 80% and 20%, respectively, of total milk proteins.

Keywords: Bioactive peptides, Caseins, Lactobacillus, Milk proteins, Whey proteins

1. INTRODUCTION

Milk proteins have been recognized as one of the most significant sources of bioactive peptides. Upon consumption, peptides with potent physiological activities may be liberated from milk proteins by the action of proteolytic enzymes in the gut and thus influence the major body's systems including endocrine, nervous, digestive, cardiovascular and immune systems (Pihlanto, 2006). Möller et al (2008) defined bioactive peptides as substances that can affect the biological processes of the body functions with beneficial effects. Dziuba and Darewicz (2007) reported that bioactive peptides are protein sequences that remain inactive in the native protein primary structure, but when released, for example by proteolytic enzymes, may regulate the most body's physiological functions. Bioactive peptides have been isolated from many protein sources such as soy proteins, gelatine, fish proteins; and maize, but milk proteins appear to be the most important

sources of bioactive peptides identified thus far. Milk proteins have been recognized as potential sources of biological active peptides that are latent and encrypted in their native form. These biologically active peptides can be generated and activated by different mechanisms including: (a) protein hydrolysis by digestive enzymes (b) food processing and (c) proteolytic activity by enzymes derived from microorganisms, especially lactic acid bacteria. Potent biologically active peptides have been isolated from a number of fermented dairy products such as cheese, fermented milk and yoghurt. Due to growth requirements, dairy starter cultures have developed highly sophisticated proteolytic system capable of breaking down milk proteins, mainly αs1- and β-caseins. The lactic acid bacteria (LAB) proteolytic structure and their activities in dairy products including yoghurt and cheese have been studied extensively (Korhonen and Pihlanto, 2006).

Milk fermented by LAB has been known since thousands of years to preserve milk for prolonged storage. In addition to the preservation role from spoilage, fermented milk has been recognized to have other functionalities for human health. Degradation of milk proteins during fermentation is a potential means to improve their nutritional value for both humans and animals. Recently, a great attention has been paid to milk protein hydrolysis as potential ingredients to health-promoting functional foods targeting diet-related chronic diseases, such as cardiovascular disease, diabetes mellitus type 2 and obesity (Korhonen, 2009). Rachid et al., (2006) reported that a diet rich in cultured dairy products may inhibit the proliferation of many cancerogenous cells. The same author also stated that the epidemiological studies had suggested that the oral intake of LAB dairy products may minimize the incidence of colon cancer. Similarly, Mensink (2006) reported that the consumption of skimmed fermented dairy products such as yoghurt was associated with reducing the risk of development of type 2 diabetes. It has also been reported that there was a relationship between low fat dairy products consumption and the possibility of reducing the overweight syndrome. Furthermore, oral administration of milk and milk products has been linked with the reduction of hypertension. All these health beneficial effects may be due to the biological compounds derived from milk proteins hydrolysis and other effectors, such as the weight control effects of milk calcium. These protein-derived compounds known as bioactive peptides may exert a number of activities affecting the digestive, endocrine, cardiovascular, immune and nervous systems under *in vitro* and *in vivo* conditions (Korhonen, 2009).

Bioactive peptides were first reported in 1950 when casein-derived phosphorylated peptides enhanced vitamin D-independent calcification in rachitic infants upon ingestion (Hayes et al., 2007). Fitzgerald and Murray(2006) defined bioactive peptides as ‘peptides with hormone- or drug-like activity that eventually regulate physiological function through binding interactions to specific receptors on target cells leading to induction of physiological responses’. In recent years, a number

of *in vitro* studies has been provided evidence for the existence of biological active peptides and proteins derived from foods that might have beneficial effects on human health. These primary studies have opened a new scientific field to examine the production of bioactive peptides from many types of dietary proteins. These biologically active peptides are hidden in their parent protein sequence and can be released by gastrointestinal tract (GIT) enzymes, food processing and fermentation. Various health benefits including anticarcinogenic, weight management, antithrombotic, antioxidative, immunomodulatory and antihypertensive properties, have been reported (Korhonen, 2009).

2. SOURCES OF BIOACTIVE PEPTIDES

In addition to milk proteins, as an important source of bioactive peptides, plants such as wheat, maize, soy, rice, mushroom, pumpkin and sorghum, as well as meat, fish, eggs from animals have been identified as other sources of bioactive peptides. Milk as a complete diet for infants consists of critical nutritive elements including lactose, fat and proteins, required for their growth and development. Milk proteins are the most important constituents of milk due to their nutritional, physiological and functional properties, which are extensively used in the food industry (Möller et al., 2008).

3. MILK PROTEINS

Caseins

In all mammals, milk caseins are a family of phosphoproteins. They exist in milk as complex micelles of the proteins and mineral calcium phosphate. About 80% of total milk proteins are casein proteins in bovine, ovine, caprine, and buffalo milk. αs1- and αs2-caseins (CN), ß-CN and κ-CN are the principal casein fractions. Moreover, bovine caseins contain minor proteins as a result of limited proteolysis by plasmin. The action of plasmin on αs1-CN and β-CN produces λ-caseins and γ-caseins and proteose peptones, respectively (Fox and Brodkorb, 2008). The isoelectric point of casein is 4.6. Casein has a negative charge in milk at pH 4.6. The purified protein is not soluble in water. Even though it is insoluble in neutral salt solutions as well, with dilute alkalis and salt solutions such as sodium oxalate and sodium acetate, it is readily dispersible. It is important to note that many distinguishing properties of casein proteins are based on their charge distribution and as well as their sensitivity to calcium precipitation within the group of caseins. Most of milk caseins exist in a colloidal particle recognized as the casein micelle. The biological function of the casein micelle is to convey amounts of highly insoluble colloidal calcium phosphate (CCaP) to all mammalian young in liquid form and to form a clot in the stomach for required nutrition.

Moreover, the micelle also contains enzymes such as lipase and plasmin enzymes, in addition to citrate, minor ions, and entrapped milk serum (Fox and Brodkorb, 2008).

Whey Proteins

In bovine milk, whey proteins comprise of four main types of proteins including β -lactoglobulin (β - Lg, 50%), α-lactalbumin (α-La, 20%), blood serum albumin (BSA, 10%), Lactoferrin (Lf) and immunoglobulins (Ig, 10%; mainly IgG1, with lesser amounts of IgG2, IgA and IgM). In human milk, there is no β -Lg and the principal Ig is IgA. The principal whey proteins are well characterized (Ha and Zemel, 2003). Whey proteins own secondary, tertiary and in most cases, quaternary structures in high levels. It has been reported that whey proteins are typical globular proteins and denature upon heating e.g. at 90°C for 10 min. Whey proteins are also not phosphorylated and insensible to Ca++ (Fox and Brodkorb, 2008). They have the most important biological role, such as carrying of calcium, zinc, copper, iron and phosphate ions in the body. They also play a biological activity as an important source of a number of different bioactive peptides (Korhonen and Pihlanto, 2006). Dropping pH at 4.6 by acidification or rennet coagulation allows keeping whey proteins in solution. However, other methods such as ultra-centrifugation, gel filtration as well as membrane technologies are ways that can be used to separate whole caseins from whey proteins (Léonil et al., 2000).

4. PHYSIOLOGICAL FUNCTIONS OF DAIRY DERIVED BIOACTIVE PEPTIDES

Fermented dairy foods, in addition to providing energy and nutrients, also are a source of physiologically important peptides that have a positive impact on body's functions. These potential health benefits may be due to the production of microbial metabolites, such as cell wall components, bacteriocins and the hydrolysis of cell-free extracts containing proteinase and peptidase activities on milk proteins substrates (Hernández et al., 2005). Research carried out during the last 10-15 years has shown that the caseins and whey proteins can be an important source of biologically active peptides or bioactive peptides. Bioactive peptides are described as 'food derived components that in addition to their nutritional value exert a physiological effect in the body'. Bioactive peptides usually contain 3 to 20 amino acid residues per molecule. They have been found to have specific activities, such as antihypertensive, antioxidative, antimicrobial, immunomodulatory, opiod or mineral-binding activities. Many milk-derived bioactive peptides reveal multifunctional properties, i.e., specific peptide sequences may exert two or more different biological activities. Due to their physiological and physicochemical versatility, milk-borne bioactive peptides are regarded as important ingredients for health-promoting functional foods (Korhonen and Pihlanto, 2006).

Fitzgerald and Meisel (2003) reported that some of the well-recognized activities of known bioactive peptides are antihypertensive and immunomodulatory activities. Moreover, risks of acquiring some of chronic diseases and metabolic disorders that are associated with unbalanced diet may be reduced by consumption of fermented dairy products. Some of the explored risk lowering effects involved cancer, osteoporosis, coronary heart diseases, hypertension and obesity.

5. CONCLUSION

Fermented dairy products and other foods containing bioactive peptides would appear to have the potential to offer specific health benefits to consumers. While there is a need for further basic research to clarify why these peptides have physiological effects, commercial products containing bioactive peptides are now commercially available. Food and pharmaceutical companies are actively considering how to exploit bioactive peptides in both human nutrition and in health promotion. Bioactive peptide preparations have the potential to be used in the formulation of functional foods, cosmetics and as potent drugs having well defined pharmacological effects. With the rise of consumer concerns about the deleterious effects of chemical preservatives and the increasing preference for natural components, milk derived bioactive substances may have value in food preservation and nutraceuticals.

Application of enrichment protocols such as membrane processing and chromatographic isolation may also be an area of future interest in the extraction of potent biofunctional peptides from fermented dairy products and their subsequent utilization as functional food ingredients. Molecular studies are required to study the mechanisms by which the bioactive peptides exert their activities. Ultimately this research may be helpful in understanding, preventing and treating life-style related diseases such as cardiovascular disease, cancers, osteoporosis, stress and obesity.

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