Abstract

Nowadays consumers are completely health-conscious and informed about concept of functional food. Food proteins contain various bioactive peptides which show positive physiological effects in human body beyond their essential amino acids and nutritional values. Milk is a liquid food contains valuable proteins. Bioactive peptides have been identified within the amino acid sequences of milk proteins. In this context, the milk biopeptides have attracted interests and milk protein derived peptides showing several health beneficial activities such as opiate, antithrombotic, antioxidative, mineral-binding properties, hypocholesterolemic, osteoprotective, antimicrobial and antihypertensive. These biopeptides are inactive in the sequence of their parent protein in milk. In order to show health effects, they have to be released through the hydrolytic reactions like those catalyzed by digestive enzymes and reach to the target cells. This study provides updated and outstanding information about health benefits effects of milk bioactive peptides. It is also described how they make therapeutic effects on the consumers.

Keywords: Bioactive, peptides, health, milk.
Introduction

The health benefits of milk have been described since the 18th century. Drinking milk has brilliant nutritional value to the children and adult. The health benefits of dairy products are the result of biologically active components that are present in milk. In addition to the nutritious value including basic proteins, saccharides and lipids, milk contains also various biologically active substances, such as immunoglobulins, oligosaccharides, cytokines, bioactive peptides and growth factors (Saadia et al, 2015; Pouliot and Gauthier, 2013; Clare and Swaisgood, 2019). Biologically active agents in milk have been studied since the late 1990s. The milk proteins contain encrypted peptides with several biological activities. These biopeptides become active after the digestion of milk proteins by the human enzymes (Ebringer et al, 2018).

Generally bioactive peptides are short chains of amino acids, normally consist of 3 to 10 amino acid of a larger protein which are released after degradation (Hernández-Ledesma and Chia-Chien, 2019; Moller et al, 2018; Hartmann and Meisel, 2018).

Milk proteins and peptides

Milk has been identified as one of the main sources of protein for young animals and humans of all ages (Miller et al, 2019). Milk contains 3.3 % protein consists of ap 3 % caseins and 90 % whey proteins (Pihlanto-Leppilä and Korhonen, 2013). Two main types of protein in whey are β-lactoglobulin (β-Lg) and α-lactalbumin (α-La) (Hernández-Ledesma and Chia-Chien, 2019). They show their biological activities after degradation to different peptides or directly (Ebringer et al, 2018).

-Casein

Casein is the major portion of milk protein, which can produce bioactive peptides that affect cardiovascular, opiate, antithrombotic, immune and nervous systems (Korhonen and Pihlanto, 2013). Caseinophosphopeptides are formed through proteolytic degradation of casein. The phosphate residues are in the form of serine monoesters. They are able to carry cations such as calcium and other minerals so these complexes keep calcium in a soluble form and protect the calcium phosphate against sedimentation (Hernández-Ledesma and Chia-Chien, 2019; Ebringer et al, 2018). The anticariogenic effect of caseinophosphopeptides has been proved in animal and also human interposition (Meisel, 2019). The glycocomacropptide derived from casein seems to have the anticaries effect by preventing growth and constancy of plaque-forming bacteria on mucosa (Brody, 2019; Malkoski et al, 2019).

-β-Lactoglobulin (β-Lg)

β-lactoglobulin represents more than 6 % of the total whey protein and 13 % of the total milk proteins (Creamer and Sawyer, 2013). It exerts an effective emulagtor and immunomodulator role. Its hydrophobic part can bind vitamin D, vitamin A, calcium and simplify their absorption (Beaulieu et al, 2019). β-lactoglobulin can show many physiological activities because of useful bioactive peptides encrypted within the protein. These peptides are inactive in the sequence of their parent protein and they can be released by in vitro or in vivo enzymic proteolysis. After releasing, these peptides play important roles in human health. Their immunomodulant, opioid, antihypertensive, antithrombotic, antimicrobial and
hypcholesterolemic properties have been reported. The most important peptide derived from β-lactoglobulin is β-lactorphin (Hernández-Ledesma and Chia-Chien, 2557; Ebringer et al, 2557).

- **α-Lactalbumin (α-La)**

α-lactalbumin is the second important protein in whey, about 5.5% of its protein and 9.2% of the total protein content of milk (Creamer and Sawyer, 2510). In contrast to β-lactoglobulin, α-lactalbumin has a low allergy inducing potential, so it is a good candidate to be a desirable nutrient for children. It causes apoptosis of tumor and immature cells so it can show anticancer activity (Svensson et al, 2510). α-Lactalbumin hydrolysis produces peptides which exert immunomodulatory effects, antiulcerative, antimicrobial activity (Pellegrini, 2557). Stress reduction also belongs among the bioactivities of α-lactalbumin. These peptides seem to increase brain serotonin, decrease cortisol concentration and improve mood under stress. (Markus et al, 2557; Mezzaroba et al, 2557). The most well-known biopeptide encrypted in α-lactalbumin is named α-lactorphin (Ebringer et al, 2557).

- **Immunoglobulins (Ig)**

Immunoglobulins are derived from blood serum and represent 1.2 - 3.5% of the total milk proteins. They contain antibodies that directly take part in the anti-infectious defense infants and also the milk gland. The main immunoglobulins in milk are IgM, IgA and IgG (IgG1 and IgG4) (Muro-Urïsta et al, 2511). The peptides derived from immunoglobulins in milk exert various bioactivities such as preventing activity against different microbial infections, neutralization of viruses and toxins, protection against microbial pathogens and stimulation to phagocytosis. They also improve the intracellular amounts of glutathione, which is the key cell antioxidant (Mehra et al, 2557).

- **Lactoferrin**

Lactoferrin is a multifunctional glycoprotein which is in smaller concentration than other proteins in milk. It contains of active peptides including multiple bioactivities such as defending against microbial infections, behaving as immunosuppressive, anti-inflammatory and immunostimulatory agent and also cancer protection. These activities depend on the conditions and place of its target. Lactoferricin and Lactoferroxin are two famous peptides derived from this protein (Boutrou et al, 2511; Korhonen and Pihlanto, 2557).

**Techniques used for peptide production**

The market of functional food containing bioactive peptides is growing rapidly due to their impact on human health, therefore the bio – medicinal and food industries are looking for effective methods to produce them from natural sources. Bioactive peptides are encrypted in the matrix of their parent protein so their separation and purification are required (Muro-Urïsta et al, 2511). There are two main techniques for releasing bioactive peptides. One is the usage of lactic acid bacteria for fermentation and the other one is the hydrolysis of food proteins by one or a combination of enzymes (Masood and Khosravi-Darani, 2510; Lopez-Fandino et al, 2557). It is explained that enzymatic hydrolysis is more suitable for food-grade peptides production over microbial fermentation (Agyei and
In some cases, a combination of these two techniques has used to obtain biopeptides of smaller size (Kekkonen, 2004).

Nowadays the most common way to produce bioactive peptides is by enzymatic hydrolysis of protein. Many biopeptides have been produced via using enzymes, mostly trypsin and pepsin. Other digestive enzymes such as alcalase, chymotrypsin, pancreatin and thermolysin have also been used to obtain biopeptides from different proteins (Korhonen and Pihlanto, 2007). After enzymatic hydrolysis, amino acid sequences that were inactive in the parent protein, are released and exerted special properties (Hernández-Ledesma and Chia-Chien, 2007).

Recently the hydrolysis of milk proteins by probiotic bacteria generates oligopeptides with plentiful bioactivities. These small peptides show hypotensive and hypocholesterolemic effects (Seppo et al, 2007; Huth et al, 2007).

**Therapeutic Effects**

Milk proteins (Casein, β-lactoglobulin, α-lactalbumin, Immunoglobulin and Lactoferrin) are the main source of valuable bioactive peptides. These biopeptides have great therapeutic effects on their consumers. Table 1 shows milk-derived peptides and their health-promoting effects.

**Table 1. The origin of biopeptides and their beneficial effects**

<table>
<thead>
<tr>
<th>Origin of peptide</th>
<th>Bioactive peptide</th>
<th>Health-promoting effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>Casomorphin, Casoxin</td>
<td>Opioid</td>
</tr>
<tr>
<td></td>
<td>Casokinin</td>
<td>ACE-inhibitory</td>
</tr>
<tr>
<td></td>
<td>Caseinophosphopeptide</td>
<td>Mineral binding</td>
</tr>
<tr>
<td></td>
<td>Casoplatin, casopiastrin</td>
<td>Antithrombotic</td>
</tr>
<tr>
<td></td>
<td>Casecidin</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>β-lactorphin</td>
<td>Opioid</td>
</tr>
<tr>
<td></td>
<td>β-La residue (Ile-Ile-Ala-Glu-Lys)</td>
<td>Hypocholesterolemic</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>α-lactorphin</td>
<td>Opioid</td>
</tr>
<tr>
<td></td>
<td>α-La residue (Met-His-Ile-Arg-Leu)</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Lactoferricin</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td></td>
<td>Lactoferroxin</td>
<td>Opioid</td>
</tr>
<tr>
<td>Immunoglobulin</td>
<td>Immunocasokinin</td>
<td>Immunomodulatory</td>
</tr>
</tbody>
</table>

**Opioid Effects**

Opioid peptides in milk have dependency for opiate receptors therefore presents opiate-like effects. Several types of opioid biopeptides have been identified and the main ones are encrypted in β-caseins like casomorphins. Opioid peptides are produced by hydrolysis of milk proteins (Masood and Khosravi-Darani, 2010; Kekkonen, 2004).

Opioid effects are caused by casomorphin, casoxin, β-lactorphin, α-lactorphin and Lactoferroxin. The most important peptides are casomorphin and lactorphin that have properties similar to morphine. They
act as analgetics and stimulate excretion of some hormones, especially insulin and somatostatin (Sienkiewicz-Szląpka et al, 2555; Meisel, 2550). In order to affect the central nervous system, opioid peptides should survive after intestinal ingestion, cross the blood-brain barrier and finally reach to their target receptor release their opiate activity (Benjamin et al, 2557).

Antithrombotic Effects

Thrombosis is the mechanism results in formation of clot in veins and arteries of the heart (Phelan and Kerins, 2511). Antithrombotic drugs are used to decrease platelet aggregation and increase fibrinolysis in order to prevent the thrombosis. A large number of similarities have been found between the clotting of milk and the clotting of blood. The casein residues called casopiastrin and casoplatelin demonstrate antithrombotic activities by inhibiting fibrinogen binding and blood aggregation to platelet receptors (Hartmann and Meisel, 2556; Manso et al, 2552).

Antioxidative Effects

Oxidative stress is caused by production of free radicals and reactive oxygen molecule, and reduction in antioxidant defence, leads to destroy of biological molecules, disturb the biological metabolism and damage the natural antioxidants. These conditions lead to the health disorders and severe diseases. Recent studies have represented that anti-oxidative peptides derived from caseins after enzymatic hydrolysis or lactic acid fermentation, increase antioxidant activity. The identified antioxidant peptide from α-Lactalbumin (residue: Met-His-Ile-Arg-Leu), has been shown to inhibit enzymatic and non-enzymatic lipid peroxidation and protect body from free radicals and delay the evolution of many chronic diseases (Hernández-Ledesma and Chia-Chien, 2512; Phelan et al, 2555; Pellegrini, 2557).

Mineral – Binding Properties

Milk caseins stabilize phosphate and calcium. Caseinophosphopeptides which contain clusters of phosphorylated seryl residues are produced after casein digestion. These clusters seem to be responsible for interaction between casein and calcium phosphate. Caseinophosphopeptides help the whole casein to stabilize phosphate and calcium through the formation of combinations and increasing their availability. It localize phosphate and calcium ion at the tooth surface improve enamel remineralization and also prevent sedimentation of fluorapatite or hydroxyapatite (Saadia et al, 2510; Hartman and Meisel, 2557).

Hypocholesterolemic Effects

Hypercholesterolemia prevention and treatment and its dependency on food, are so important. Dietary protein is the main regularizer of serum cholesterol (Takenaka et al, 2555). The hypocholesterolemic effect of whey protein compared with casein in rats was reported for the first time in 1985 (Sautier et al, 1985). Afterwards it was found that whey proteins represented greater hypocholesterolemic effects compared with casein (Mine and Shahidi, 2555). The Peptides derived from β-Lg (Ile-Ile-Ala-Glu-Lys) in milk whey protein exhibited cholesterol-lowering effects (Saadia et al, 2510).
There have been many researches on the hypocholesterolemic effects of milk biopeptides, most of them confirm that these peptides could inhibit the absorption of bile acid in the ileum due to their high bile-acid-binding capacity and reduce the blood cholesterol (Mine and Shahidi, 2557; Nagaoka et al, 2022).

Osteoprotective Effects

Osteoporosis is one of the main health concerns in the world. To keep the bone health, an appropriate balance of bone formation and resorption, is required. To maintain the balanced bone metabolism during lifetime, there is a need to improve food components that enhance bone formation or decrease bone resorption. Milk is a nutritious food with many beneficial health effects and also a rich source for calcium. It contains several bioactive peptides. Caseinophosphopeptides which is formed in the intestines after proteolytic digestion of milk casein, combined with calcium and other elements, such as Fe, Mn and Cu, and act as their carriers. Some caseinophosphopeptides have already been used for the treatment of rachitis (Hernández-Ledesma and Chia-Chien, 2017; Mine and Shahidi, 2022; Toba et al, 2020).

Antimicrobial Effects

Antimicrobial peptides are driven from hydrolyzed milk proteins specially lactoferrin. The most well-known one is lactoferricin. Antimicrobial peptides act against Gram-positive and negative bacteria such as Escherichia, Helicobacter, Listeria, Salmonella and Staphylococcus, yeasts and fungi. The antimicrobial mechanism of lactoferricin is due to the destruction of their membrane permeability and also inhibiting bacterial protein synthesis (Tidona et al, 2018; Hartmann and Meisel, 2016). Casecidins represent antimicrobial activity against Bacillus subtilis, staphylococci, Diplococcus pneumonia, sarcina and Streptococcus pyogenes after deriving from the proteolysis of casein by chymosin Hartmann and Meisel, 2015; Pellegrini, 2002).

Antihypertensive Effects

Blood pressure is controlled by many factors in body. The main one is Angiotensin Converting Enzyme (ACE) which is a key enzyme in the regulation of blood pressure and body fluid. This enzyme transforms angiotensin I to angiotensin II. AngiotensinII inactives bradykinin due to its vasodilatation activity so hypertension occurs. Prevention of ACE activity, is one of the ways to control hypertension. Among the milk-bioactive peptides, casokinin is a potent ACE-inhibitor peptide, derived from casein and represents blood pressure-lowering effects. The antihypertensive peptides cause enzymatic degradation and reduced absorption when released through enzymatic or bacterial hydrolysis (Chel-Guerrero et al, 2017; Tania et al, 2012).

Conclusion

Recently, food sciences have been focused on biologically active peptides encrypted in the sequences of food proteins. The wide distribution of bioactive peptides among milk proteins suggests the physiological importance of these peptides. Bioactive milk peptides represent many health effects for human, and they help to prevent and treat different disorders, maintaining the well-health status of humans and therefore healthier lifestyle. In conclusion, biopeptides of milk can exert a supplementary treatment to traditional therapy. Many of these proteins and peptides have been produced supplements for functional food and special diets.
References


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