Asian J. Adv. Basic Sci.: **2017,** **5***(2),* 154-167
ISSN (Print): 2454 – 7492
ISSN (Online): 2347 – 4114
www.ajabs.org

Fermentable Bioactive Peptides as a Functional Molecule

Tariq Ahmad Ganaie[1*], Huma Mukhtar[2], Farhana Mehraj Allaie[3] and Shaiq Ahmad Ganie[4]

[1, 2, 3 & 4]Department of Food Technology, IUST Awantipora - 192122, INDIA

[*] Correspondence: E-mail: tariqtech@gmail.com

(Received 22 Sept, 2017; Accepted 29 Dec, 2017; Published 31 Dec, 2017)

ABSTRACT: Bioactive peptides are peptides with biological activity that have positive impact on physiological functions of body and play a vital role in human health. They have drug like activity and are used to produce neutraceuticals and functional foods. Food sources of bioactive peptides are milk, soybean, pulses, fish, meat, egg, wheat etc. These peptides are produced by enzymatic hydrolysis with digestive enzymes and microbial activity of fermented foods and proteolysis by enzymes. Microbial fermentation is efficient way to produce these peptides. Physiological functions (health benefits) of bioactive peptides include antihypertensive, antioxidative, antithrombotic, hypocholesterolemic, opioid, mineral-binding, antimicrobial, immuno and cyto-modulatory, anti-cancer, anti-HIV and nutritional.

Keywords: Bioactive peptide; food sources; microbial fermentation and physiological functions/health benefits.

INTRODUCTION: Bioactive peptides(BAP's) have been defined as specific protein fragments that have a positive impact on body functions and conditions and may ultimately influence health (Kitts; Weiler, 2003). According to Fitzgerald and Murray (2006), bioactive peptides have been defined as peptides with hormone- or drug like activity that eventually modulate physiological function through binding interactions to specific receptors on target cells leading to induction of physiological responses. According to their functional properties, bioactive peptides have classified as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding and antioxidative. These peptides play an important role in human health. Bioactive peptides generally contain 3–20 amino acid units (Pihlanto-Leppala, 2000), but in some cases this range is extended. Lunasin, for example, is a food-derived peptide with anticancer activity, composed of 43 amino acids with a molecular weight (MW) of 5400 daltons (JEONG et al., 2002). Biologically active peptides, once liberated as independent entities, act as potential metabolism modulators and regulatory compounds with hormone-like activities (Korhonen; Pihlanto, 2003).

BAP's are food protein derived peptides that posses beneficial pharmacological properties beyond normal and adequate nutrition (Hartmann; Miesel, 2007). The food processing steps lead to concentration of the active peptides with the enhancement of the physiological activity of the products, which could also be nutritionally beneficial as a source of essential amino acids. This approach can provide the opportunity for diversification of the use of agricultural crops and animal products beyond basic nutritional purposes, especially as a source of active ingredients for formulation of food products with health benefits.

Fermented milk products have naturally high nutritional value, and have many health-promoting effects, such as improvement of lactose metabolism, reduction of serum cholesterol and reduction of cancer risk. The beneficial health effects associated with some fermented dairy products may, in part, be attributed to the release of bioactive peptide sequences during the fermentation process. Numerous peptides and peptide fractions, having bioactive properties have been isolated from fermented dairy products. Many recent articles and book chapters have reviewed the release of various bioactive peptides from milk proteins through microbial proteolysis (Takano, 2002; Korhonen; Pihlanto, 2004; Fitzgerald; Murray, 2006; Jakala; Vapaatalo, 2010).

Bioactive peptides are included in the group of protein ingredients which produce functional foods (NAGPAL et al., 2011). Within the group of bioactive peptides, the ones that have attracted higher scientific and industrial interest are those having antihypertensive activity. Interest in studying these compounds has arisen from the increase in mortality in industrialized countries due to hypertension and/or renal, heart or brain complications (Rojas-Ronquillo Et Al., 2012; Nielsen Et Al., 2009; Hartmann; Meisel, 2007; Wu; Ding, 2001; Takano, 1998). Components of proteins in marine foods also contain sequences of bioactive peptides, which could exert a physiological effect in the body. Especially, some of these bioactive

peptides have been identified to possess nutraceutical potentials that are beneficial in human health promotion. Moreover, the possible roles of marine food-derived bioactive peptides in reducing the risk of diseases have been reported. (Erdmann et al., 2008; Lee; Maruyama, 1998).

Sources of bioactive peptides: Numerous animal and plant food proteins have been exploited as sources of BAPs. Several studies on BAPs were conducted using animal proteins mostly milk proteins, casein and whey, egg and meat muscle proteins have also yielded BAPs. In addition, several BAPs have been produced from marine protein sources, including fish, salmon, oyster, macro algae, squid, sea urchin, shrimp, snow crab, and seahorse. Typical plant food proteins used for the production of BAPs include soy, pulses (lentil, chickpea, pea, and beans), oat, wheat, hemp seed, canola, and flaxseed. Based on the current literature, food proteins are selected as sources of BAPs based on 2 major criteria (1) a pursuit of value-added use of abundant underutilized proteins or protein-rich food industry by-products, and (2) utilization of proteins containing specific peptide sequences or amino acid residues of particular pharmacological interest.

Dairy products: Milk particularly is a rich source of bioactive peptides. These peptides are present in both casein (Alpha, Beta, kappa, gamma-casein) and whey proteins (alpha-lactalbumin, beta-lactoglobulin, lactoferrin, and immunoglobulins) (Gobbetti et al., 2002; Belem et al., 1999) and can be released by enzyme hydrolysis or microbial fermentation. For enzyme hydrolysis, single enzymes or a combination of proteinases have been used, such as trypsin, alcalase, chymotrypsin, carboxypeptidase, pancreatin, pepsin, and enzymes from bacterial or fungal origin (for example, proteinase K from *Tritirachium album*). Bioactive peptides have been identified from milk fermented by lactic acid bacteria, such as *Lactococcus lactis subsp.cremoris, Lactobacillus helveticus, Lactobacillus GG strain, Lactobacillus delbruskii subsp.Bulgaricus* (Gobbetti et al., 2002; Leblanc et al., 2002; Korhonen; Pihlanto, 2003).

Egg: Several bioactive peptides with vasodilatation, ACE-inhibitory activities have been found in egg ovalbumin treated with chymotrypsin and pepsin (Korhonen; Pihlanto, 2003).

Meat: Chicken meat was found to be a source of antihypertensive peptides. For example, with the action of the enzyme thermolysin, 2 antihypertensive, Ile-Lys-Trp and Leu-Lys-Pro have been detected (Korhonen; Pihlanto, 2003).

Fish and Sea foods: Bioactive peptides can also be found in fish products, such as sardine muscle, tuna muscle, bonito (Yamamoto et al., 2003) and Alaska Pollack skin (Korhonen; Pihlanto, 2003).

Insects: Royal jelly (RJ), a bee product rich in proteins, has also been found as a good source of ACE-inhibitory peptides. Matsui et al (2002) found that intact RJ and its protein fraction did not show ACE-inhibitory activity. However, after pepsin and the subsequent trypsin and chymotrypsin hydrolysis, ACE inhibitor capacity was developed.

Soybean: Soybean-based foods contain an array of biologically active compounds that can confer important health benefits such as antioxidant effects (Setchell, 1998; Tsai; Huang, 1999). These phytochemicals include saponins, phytates, protease inhibitors, phenolic acids, and lecithin, all known for their anticancer potential (Cohen et al., 2000; Messina; Flickinger, 2002); phytosterols, which have hypocholesterolemic effects; isoflavones, which are known for several health benefits (Fukui et al., 2002) and omega-3 fatty acids which have well recognized cardioprotective effects. Among these compounds, isoflavones have attracted the most attention (Messina; Flickinger, 2002; Messina; Loprinzi, 2001).

Production of bioactive peptides: There are a number of methods by which peptides with biological activity can be produced from precursor proteins. The most common ones are

(a) Enzymatic hydrolysis with digestive enzymes,
(b) By means of the microbial activity of fermented foods, or
(c) By the action of enzymes derived from proteolytic micro-organisms.

Production by microbial fermentation: Fermentation is considered to be an efficient way to produce bioactive peptides. Bioactive peptides can be released by the microbial activity of fermented food or through enzymes derived from microorganism (Korhonen; Pihlanto, 2003). Fermented milk and cheese have been extensively studied to investigate their potential to form bioactive peptides. Interest in fermented soybean products, such as natto, tempeh, soy sauce, soy paste, has grown in recent years. Many bioactive peptides have been identified. For example, ACE-inhibitory peptides containing Ala, Phe, and His have been isolated from soybeans fermented by *Bacillus natto* and chunggugjang fermented by *Bacillus subtilis* (Korhonen; Pihlanto, 2003). ACE-inhibitory peptides were also found in soybean paste (His-His-Leu) (SHIN et al., 2001), soy sauce (Okamoto et al., 1995), natto, and tempeh (Gibbs et al., 2004).Three potent ACE-inhibitors, 3 thrombin inhibitors, 5 peptides with surface-active properties, and 1 peptide with antibacterial activity were also found in enzymatic hydroly-

sates of soy fermented foods. They were all derived from glycinin, while -conglycinin was found more stable to proteolytic attack even by multi-enzyme preparations (Gibbs et al., 2004).

Many industrially utilized dairy starter cultures are highly proteolytic. Bioactive peptides can, thus, be generated by the starter and non-starter bacteria used in the manufacture of fermented dairy products. The proteolytic system of lactic acid bacteria (LAB), e.g. *Lactococcus lactis, Lactobacillus helveticus and Lb. delbrueckii ssp.bulgaricus*, is already well characterized. This system consists of a cell wall-bound proteinase and a number of distinct intracellular peptidases, including endopeptidases, aminopeptidases, tripeptidases and dipeptidases (Christensen et al., 1999). Rapid progress has been made in recent years to elucidate the biochemical and genetic characterization of these enzymes. The fact that the activities of peptidases are affected by growth conditions makes it possible to manipulate the formation of peptides to a certain extent (Williams et al., 2002). Many recent articles and book chapters have reviewed the release of various bioactive peptides from milk proteins through microbial proteolysis (Gobbetti et al., 2004; Gobbetti et al., 2002; Korhonen; Pihlanto, 2001, 2004; Matar et al., 2003). Most of these studies report the production of ACE-inhibitory or antihypertensive peptides, and immunomodulatory, antioxidative and antimicrobial peptides have also been identified. *Lb. helveticusis* widely used as a dairy starter in the manufacture of traditional fermented milk products, such as Emmental cheese and highly proteolytic *Lb. helveticus* strains capable of releasing ACE-inhibitory peptides, in particular, have been demonstrated in several studies. The best known ACE-inhibitory peptides, Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP), have been identified in milk fermented with *Lb. helveticus* strains (Nakamura et al., 1995, Sipola et al., 2002). Pihlanto-Leppala et al., (1998), studied the potential formation of ACE-inhibitory peptides from cheese whey and caseins during fermentation with various commercial dairy starters used in the manufacture of yoghurt, ropy milk and sour milk.

Microbial enzymes have been used successfully for the production of bioactive peptides from milk proteins. Yamamoto et al., (1994) reported that casein hydrolyzed by the cell wall-associated proteinase from L. helveticus showed antihypertensive activity. Several ACE inhibitory and one antihypertensive peptide were isolated from the hydrolysate. Using the same proteinase, Maeno et al., (1996) identified a beta -casein-derived antihypertensive peptide from the casein hydrolysate. This peptide did not show a strong ACE-inhibitory activity as such, but a corresponding synthetic hexa-peptide—deleted by Gln (Lys-Val-Leu-ProVal-Pro) - exhibited strong ACE-inhibitory activity as well as a significant antihypertensive effect in SHR(Spontaneously Hypertensive Rat). It was suggested by these researchers that both the proline residue in the C-terminus and the amino acid sequence might be important for ACE-inhibition of the hexapeptide. Proline is generally known to be resistant to degradation by digestive enzymes.

Synthesis of bioactive peptides: Peptides synthesis is a useful method to prepare bioactive peptides in large scale and also to study their mechanism of action. At present, 3 main approaches are available:

(1) Chemical synthesis,
(2) Recombinant DNA technology, and
(3) Enzymatic synthesis (GILL, 1996).

Chemical synthesis is the most widely used approach at laboratory scale, existing 2 variants, liquid-phase and solid phase. The solid phase approach is the most powerful method for synthesis of peptides composed of about 10 to over 100 residues on a small scale (most practical for sequences of intermediate lengths). However, the high cost of the instrumentation and reagents has largely restricted its use. On the other hand, liquid phase synthesis is the preferred method for large-scale synthesis of relatively short peptides and for carrying out the condensation of peptide fragments (Gill, 1996). Recombinant DNA technology is the preferred choice for relatively large peptides with up to several hundred amino acids (GILL, 1996). Due to the low expression efficiencies obtained and difficulties encountered in product extraction and recovery, attempts to extend this approach to the preparation of short peptides have not yet been truly successful (Korhonen; Pihlanto, 2003). Using genetic engineering techniques, Yoshikawa et al., (2002) introduced a highly potent antihypertensive peptide, into 3 homologous sites in soybean beta conglycinin alpha subunit by site-directed mutagenesis. In practice, enzymatic synthesis is currently limited to relatively short sequences.

Characterization of bioactive peptides: The isolation and purification of bioactive peptides are very important for exploration of their physicochemical properties and evaluation of their in vitro and in vivo bioactivities. Bioactive peptides can be separated from a protein hydrolysate mixture by a number of approaches, mainly, different kinds of chromatography and membrane-based separation techniques. Prior to the separation process, a peptide mixture can be subjected to ammonium sulfate precipitation, salting out, and solvent extraction in order to remove proteins, enzymes, and other components in the source materi-

al. Most chromatographic techniques for protein purification are also applicable to peptide purification, given special consideration on size difference, such as size-exclusion chromatography .HPLC is the most commonly used method for peptide separation. Membrane-based separation processes are techniques in which a component is separated from a mixture as the latter is forced through a porous membrane under applied pressure, hence the name pressure-driven membrane-based separation. They are further divided into microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), depending on the properties of the membrane used. (Shahidi; Zhong, 2008)

Health Benefits:

Antihypertensive activity: Antihypertensive peptides are probably the most extensively studied bioactive peptides from exogenous sources such as food. There has been a growing interest in antihypertensive peptides for their effectiveness in lowering blood pressure, since hypertension has become a serious health problem, occurring at increasingly high rates, especially in developed countries, and has been considered a risk factor for developing cardiovascular diseases. Antihypertensive peptides have been found effective in preventing/treating hypertension mainly by inhibiting the angiotensin-converting enzyme (ACE), which plays a key role in the regulation of blood pressure and electrolyte homeostasis (the equilibrium of water and salt in the body; Papadimitriou et al ., 2007, Tsai et al., 2008). ACE is a nonspecific dipeptidyl carboxypeptidase that converts decapeptide angiotensin I into octapeptide angiotensin II, which is known as a potent vasoconstrictor. Angiotensin II also has a regulatory effect on cellular lipoxygenases which catalyse low-density lipoprotein (LDL) oxidation, a process associated with atherogenesis (Abubakar et al., 1998). Furthermore, ACE catalyses the inactivation of the vasodilator bradykinin, which in turn leads to increased blood pressure. ACE inhibitors can decrease the activity of ACE and indirectly reduce the level of angiotensin II, thereby exerting a vasorelaxing effect on blood vessels. Clinical studies have revealed that ACE inhibitors significantly reduced the morbidity and mortality of patients with myocardial infarction or heart failure (Geisterfer et al., 1988; Daemon et al., 1991).

Cholesterol Lowering Effect: Hyperlipidemia, especially hypercholesterolemia, is one of the most important risk factors contributing to the development of cardiovascular diseases (Kannel et al., 1971). Numerous synthetic drugs and natural extracts with cholesterol-lowering effect have been explored for their potential in prevention and treatment of hypercholesterolemia. A large body of literature indicates that proteins from soybean can reduce blood cholesterol level in experimental animal models as well as in human subjects (Kim et al., 1980; Potter, 1995). An early clinical study clearly revealed that the substitution of animal proteins with soy protein resulted in a 22–25% decrease in LDL cholesterol and a 20–22% decrease in total cholesterol in hypercholesterolemic patients. The hypocholesterolemic effect of soy protein was later confirmed by more animal and clinical studies (Sagara et al., 2004; Wang et al., 2004; Adams et al., 2004; Moriyama et al., 2004; Tachibana et al., 2005), and soybean-rich diet has become the most potent dietary tool for treating hypercholesterolemia, although the mechanism has not yet been fully established. The USFDA recommended a daily intake of 25 g of soybean protein for lower level of serum cholesterol and reduction in the risk of cardiovascular disease (U.S. FOOD AND DRUG ADMINISTRATION, 1999). The hypocholesterolemic effect of soybean was first attributed to the presence of isoflavones, according to Adams et al. 2002, Zhan; HO, 2005, since ethanol-washed isoflavone-free soy protein showed less cholesterol-lowering capacity than that containing isoflavones. However, another experiment by Fukui et al. 2002 indicated that isoflavones alone did not exhibit a cholesterol-lowering effect. Hence, it was speculated that the isoflavone-protein interaction may contribute to the hypocholesterolemic effect of soy proteins (HSU, et al., 2001). More recently, it has been demonstrated that soy peptides may be responsible, at least in part, for the hypocholesterolemic property of soy proteins, based on the observation that soy protein hydrolysate showed a stronger serum cholesterol lowering effect than intact soy protein(Anderson et al., 1995; Sugano et al., 1990; Nagaoka et al., 1999).

Antioxidant Activity: Bioactive peptides which have an antioxidative effect have previously been obtained from various dietary proteins after enzymatic hydrolysis. The presence of such peptides, derived from hydrolyzed food proteins such as caseins, whey proteins, soybean, rice bran, quinoa seed protein, buckwheat protein, egg-yolk protein, porcine myofibrillar proteins and aquatic by-products proteins, has been investigated in a number of studies (Pihlanto, 2006). They are effective against enzymatic and nonenzymatic peroxidation of lipids and essential fatty acids, as free radical scavengers, in metal ions chelation and in adduct formation. The inhibition of oxidative processes is of particular importance for the survival of cells in an organism. However, undesired oxidative processes also occur in foods. The formation of free radicals results in a deterioration of food quality, for example rancid flavor, unacceptable taste, and shortening of shelf life. Proteins, protein hydrolysates, individual

peptides, and amino acids have been shown to have significant antioxidant activities. Some amino acids were found to possess strong antioxidant activity in linoleic acid and methyl linoleate model systems (Marcuse, 1962). A mixture of tryptophan and lysine was able to inhibit oxidation of butter fat (Merzametov et al., 1976). Moreover, antioxidant properties of proline in sardine oil (Revankar, 1974), methionine in vegetable oils (Sims; Fioriti, 1977), and histidine, threonine, lysine, and methionine in a sunflower oil emulsion (Riison et al., 1980) have been reported.
Protein digests have varied antioxidant activities depending on the peptide structure, i.e., size of the peptides and their amino acid sequences, which are influenced by the source of protein and conditions of the hydrolysis process involved. Soy peptides, for example, obtained from native or heated soy protein by different enzymes, such as pepsin, papain, chymotrypsin, Alcalase, Protamex, and Flavourzyme, resulted in different degrees of hydrolysis, ranging from 1.7 to 20.6% and antioxidant activity ranging from 28 to 65%, measured as inhibition against formation of thiobarbituric acid-reactive substances (TBARS) in a liposome-oxidizing system (Pena-Ramos Et; Xiong, 2002).

Antithrombotic Activity: Functional similarities between milk and blood coagulation as well as sequence homologies exist in the fibrinogen g-chain and k-casein (Jolles; Caen, 1991). Jolles et al., (1986) showed that bovine k-casein f106-f116 inhibited platelet aggregation and combined with the receptor site, consequently preventing fibrinogen binding with blood platelets. This inhibition was dependent on peptide concentration. The two smaller tryptic peptides (k-casein f106-f112 and f113-f116) exerted a much more minimal effect on platelet aggregation and did not inhibit fibrinogen binding. These peptides are referred to as casoplatelins. The behavior of k-casein f106-f116 is similar to that of the C-terminal peptide of the human fibrinogen g-chain (Fiat et al., 1989). The mechanism involved in milk clotting, defined by interaction of k-casein with chymosin bear a remarkable similarity to the process involved in blood clotting, defined by interaction of fibrinogen with thrombin (Jolles; Henschen, 1982) . The k-casein fragment named casoplatelins, obtained from tryptic hydrolysates, shows antithrombotic activity by inhibiting fibrinogen binding platelet (Jolles et al., 1986; Jolles; Henschen, 1982). These peptides are released during gastrointestinal digestion and absorbed intact into the blood, which supports the concept that they exert an antithrombotic effect in vivo. The potential physiological effects of these antithrombotic peptides have not been established, but such peptides have been detected in the plasma of newborn children after breastfeeding or ingestion of cow milk-based infant formula (Jolles; Henschen, 1982).

Opioid Activity: Short sequences of amino acids that bind to opioid receptors in brain are known as opioid receptors. They play active role in nervous system. Endogenous and many exogenous opioid agonists and antagonists have been characterized as peptides. Their binding to opioid receptors in the central nervous system as well as in many peripheral tissues has been related to a numberof physiological and path of physiological functions, including immunological functions, gastrointestinal function control reproductive mechanism control, and regulation of many central nervous functions such as stress handling, depression,and other emotional behaviors. Beta-Casomorphins from beta-casein were the first opioid peptides identified from food protein sources and have so far been most frequently studied. Peptides with opioid activity have been identified in various casein fractions hydrolyzed by digestive enzymes (Brantl et al., 1979; Pihlanto-Leppala et al., 1994; Teschemacher, 2003). These opioid peptides are opioid receptor ligands with agonistic or antagonistic activities. Opioid receptors are located in the nervous, endocrine and immune systems as well as in the gastrointestinal tract of mammals and can interact with their endogenous ligands and with exogenous opioids and opioid antagonists. Thus, orally administered opioid peptides may modulate absorption processes in the gut and influence the gastrointestinal function in two ways: first, by affecting smooth muscles, which reduces the transit time, and second, by affecting the intestinal transport of electrolytes, which explains their antisecretory properties. The actual physiological effects of milk-derived opioid peptides remain, however, to be confirmed. Beta-Casein-derived opioid peptides (beta-casomorphins) or their precursors have been detected in the duodenal chyme of minipigs, in the plasma of newborn calves and in the human small intestine upon oral administration of casein or milk (Miesel, 1998; Meisel; Fitzgerald, 2000; Meisel; Fitzgerald, 2003). Opioid casein fragments have not been detected in the plasma of adult mammals and, therefore, it is suggested that only the neonatal intestine is permeable to casomorphins. Interestingly, a as1-casein-derived peptide f (91–100) has been demonstrated to possess anxiolytic-like stress-relieving properties in animal model and human studies (Lefranc, 2001). This peptide has been employed commercially as an ingredient, e.g. for confectionery and soft drinks.

Mineral Binding Activity: Peptides derived from in vivo and/or in vitro enzymatic proteolysis of whole protein exhibit mineral-binding activity, which contri-

butes both to their antioxidant activity and mineral absorption-enhancing activity. Bioactive peptides such as caseinophosphopeptides from beta casein are able to chelate pro-oxidant metals, in particular iron and copper, and therefore protect from lipid oxidation-caused food deterioration and oxidative stress-mediated cellular damage. Copper-chelating peptides have been purified from sunflower protein hydrolysates, and their metal-chelating activity was found to be dependent on histidine content and peptide size (Megias et al., 2007). The metal-chelation property of the peptides was attributed to the imidazole ring in histidine, which is directly implicated in peptide binding to copper. The results suggested the potential antioxidant role of food-derived peptides in preventing copper-induced in vivo oxidative damage, including DNA and LDL oxidation, which are involved in atherogenesis and other chronic diseases (Megias et al., 2007). More importantly, mineral-binding peptides are effective in enhancing in vivo absorption of metals, including copper, calcium, iron, zinc, and other trace elements and therefore improving their bioavailability. Binding of copper to certain amino acids such as histidine, methionine, and cysteine in small peptides mediates absorption of copper through these amino acid transporters (Gaetke et al., 2003). Caseinophosphopeptides, which in most cases contain a serine phosphate cluster and glutamyl residues in the sequence of 3 phosphoseryl groups followed by 2 glutamic acid residues, can bind to calcium at the negatively charged side chains and form a soluble complex (MEISEL, 1998). This leads to enhancement of calcium absorption across enterocytes in the distal intestine (Rutherfurd- Markwick et al., 2005). Use of caseinophosphopeptides in preventing dental caries has also been proposed because of their role in recalcification of the dental enamel (Reynolds, 1987).

Antimicrobial Activty: The best investigated antimicrobial peptide is the fragment 17-41 of lactoferrin, more commonly known as lactoferricin. Antimicrobial peptides are effective against different bacteria and yeasts but only a few in vivo studies have been carried out to date. A protection against pathogens has been attributed to α-lactalbumin and involves the release of peptides, which support the immune function in humans. Different antimicrobiological functions have been attributed to the CMP (caseino macropeptides) that is formed during cheese manufacture or digestion from K-casein. It binds enterotoxins (*cholera* and *E.coli*), modulates the immune system response, inhibits bacterial and viral adhesion, suppresses gastric secretions and promotes bifidobacterial growth. The other fragment of alpha s1-casein, viz.f1–23, known as isracidin, has demonstrated antibiotic-type activity in vivo versus *S. aureus and Candida albicans*; it can protect sheep and cows against mastitis (Laho; Regelson, 1996). Fragments of human b-casein have also a protective effect against Klebsiella pneumoniaein mice (Migliore-Samour et al., 1989). The immune modulatory peptide derived from bovine b-casein, viz. f193–209, was shown to enhance the antimicrobial activity of mouse macrophages, which had been obtained either from germ-free or from human flora associated mice, without pro inflammatory effects (Sandre et al., 2001).All these results pertaining to animals have been obtained in vivo by injecting peptides therein; at present, no studies are yet available that provide evidence for their putative effect when ingested.

IMMUNO AND CYTO MODULATORY ACTIVITY: Immuno-modulatory peptides can modulate the proliferation of human lymphocytes, down-regulate the production of certain cytokines, and stimulate the phagocytic activities of macrophages. As a result, they can regulate the development of the immune system in newborn infants. Cytomodulatory peptides have been shown to influence the viability as well as the proliferation, differentiation, and apoptosis of different cell types. Immunomodulation involves suppression or stimulation of human immune functions. Immunomodulatory food peptides act by enhancing the functions of immune system including regulation of cytokine expression, antibody production, and ROS-induced immune functions (Hartmann; Meisel, 2007; Yang et al., 2009) For example, a tryptic digest of rice protein improved immune function by promoting phagocytosis and increasing superoxide anion production in human polymorphonuclear leukocytes (Takahashi et al., 1994). In addition, egg-derived peptides also showed immunostimulating activities and were used to increase immune functions during cancer immunotherapy (Mine; Kovacs-Nolan, 2006). Moreover, a recent work showed that oral administration of a pea protein hydrolysate to mice led to reduced NO production by activated macrophages as well as reduced secretion of the proinflammatory cytokines, tumor necrosis factor (TNF)-Alpha and interleukin (IL)-6, by up to 35% and 80%, respectively (Ndiaye et al., 2012).

Anticancer Activity: Proteins, peptides, and amino acids have been implicated in preventing the development of different types of cancer. Bowman Birk protease inhibitor (BBI), a water-soluble protein isolated from legumes and many monocotyledonous seeds, has shown anticarcinogenic activity in in vitro and animal models and is now intensively studied as a cancer chemopreventive agent in clinical trials (Armstrong et al., 2000; Meyskens, 2001). Another protease inhibitor, soybean Kunitz trypsin inhibitor,

was reported to suppress ovarian cancer cell invasion by blocking urokinase upregulation (Kobayashi et al., 2004). Bovine lactoferrin and lactoferricin from bovine milk were able to inhibit lung metastasis and angiogenesis in mice transplanted with murine melanoma, lymphoma, or colon carcinoma 26 cells (Yoo et al., 1997, Iigo et al., 1999). Lectins from mistletoe extract induced powerful anticancer effects in mice inoculated with tumor cells (Pryme et al., 2002). The anticancer activities of these proteins may, at least partially, be attributed to encrypted bioactive peptides. Numerous peptides in different sizes from various sources have been indicated to render an anticancer effect in in vivo studies. Lunasin, a novel chemopreventive peptide from soybean, has been found to suppress chemical carcinogen and viral oncogene-induced transformation of mammalian cells and inhibit skin carcinogens in mice. Lunasin is a 43-amino-acid peptide containing 9 aspartic acid residues at the C-terminus, a tripeptide arginine-glycine-aspartic acid cell adhesion motif, and a predicted helix whose structure is similar to a conserved region of chromatin-binding proteins (Galvez et al., 2001). Lunasin exhibits an inhibition effect against core histone acetylation in mammalian cells (Jeong et al., 2007,) suggesting its involvement in chromatin modification, a process implicated in cell-cycle control and suppression of carcinogenesis. In addition to lunasin, other soy protein-derived peptides have also shown promising activities for anticancer therapy. Wang et al., (2008) reported that enzymatic hydrolysates from different soy varieties inhibited the viability of cultured leukemia cells which were significantly lower than the activity of lunasin. Moreover, a lunasin-containing glutelin fraction of Amaranthus hypochondriacus, when digested with trypsin, induced programmed cell death (apoptosis) in cervical cancer cells by 30% and 38% at 1 and 5μg/mL, respectively (Silva et al., 2008). It was not reported whether the anticancer peptides were derived from lunasin primary sequence or from other protein precursors present within the fraction. A similar study also demonstrated that a soy protein-derived hydrophobic peptide fraction exhibited cytotoxicity against macrophage-like murine tumor cell line by arresting cell cycle progression at the G2/M phases (Kim et al., 2000).

Bioactive Lunasin in Current Research: Lunasin is a 43-amino acid peptide with nine aspartate residues (D) at the C-terminus, an Arg-Gly-Asp (RGD) cell adhesion motif, and a conserved region of chromatin-binding proteins (Odani et al., 1987a, 1987b). Previous studies showed that lunasin suppresses carcinogenesis triggered by chemical carcinogens and oncogenes bothin vitro and in a mouse skin cancer model (Galvez et al., 2001). Hsieh et al., (2010) recently evaluated the anticancer properties of lunasin in breast cancer by using a xenograft mouse model. The US Food and Drug Administration recommend the consumption of 25 g of soy protein per day to lower cholesterol levels and reduce the riskof heart disease (Xiao, 2008); this supplies approximately 250 mg of lunasin. Whether this dose is sufficient for chemoprevention still needs to be determined. In a human feeding trial for detecting the presence of lunasin in plasma after soy protein consumption, lunasin was found in the circulation, a key requirement for its bioactivity in the target organs (Dia et al., 2009). Recently, some studies showed that lunasin can reduce low-density lipoprotein and total cholesterol levels by directly inhibiting gene expression of 3-hydroxy-3-methyl-glutaryl-CoA reductase, which reduces cholesterol biosynthesis, and increasing low-density lipoprotein receptor expression, which enhances clearance of plasma low-density lipoprotein cholesterol (Galvez, 2001). In 2001, Galvez applied the Pichia yeast expression system for large-scale production of lunasin.

Anti-human Immunod Efficiency Virus Activity (Anti-HIV): Numerous studies have been reported that marine bioactive peptides can be used as anti-HIV components in functional foods or nutraceuticals and pharmaceuticals due to their therapeutic potential in the treatment or prevention of infectious diseases. Lee and Maruyama (Lee et al., 1998) searched for HIV-1 protease-inhibiting substances from oyster C. Gigas. Two peptides inhibiting HIV-1 protease, were isolated from the hydrolysate of oyster proteins prepared with thermolysin. These two peptides exhibited strong inhibition of HIV-1 protease at IC50 values (50% inhibitory concentration) of 20 and 15 nM, respectively, and behaved as competitive inhibitors for HIV-1 protease with Ki values of 13 and 10 nM, respectively. Lee and Maruyama (Hartmann; Meisel, 2007) have searched that the length of amino acid sequence and the presence of C-, N-terminal hydrophobic amino acids in these peptides are important for their inhibitory activity. Depsipeptides isolated from a number of marine sponges have been identified to be active as HIV inhibitors. Neamphamide A, a novel HIV-inhibitory depsipeptide obtained from marine sponge *Neamphius huxleyi*, exhibited a potent cytoprotective activity against HIV-1 infection with an EC50(50% effective concentration) of 28 nm (Hartmann; Meisel, 2007). Sponges-derived peptides are indicated as promising candidates for the design of novel strong inhibitors of viral infection.

Nutritional Activity: Some peptides are able to sequester calcium and other minerals, hence acting as

biocarriers—they are called phosphopeptides; glycomacropeptide (GMP) may also exhibit a number of nutritional features.

Caseinophosphopeptides: The term phosphopeptide was pioneered by Mellander (1950), and it means a casein-derived phosphorylated peptide which enhances vitamin D-independent bone calcification in rachitic infants. Chabance et al., (1998), have proven the occurrence of caseinophosphopeptides (CPPs) in the stomach and duodenum following milk ingestion. Recently, Meisel; Fitzgerald (2003) have reviewed the structural features and physiological potential of milk-protein-derived caseinophosphopeptides, which were also shown to exert cytomodulatory effects. Iron deficiency, a major worldwide nutritional problem, can be reduced by CPPs; in fact, binding of Fe to phosphopeptides prevents formation of high-molecularweight ferric hydroxides, which are poorly absorbed. In vitro studies with rats (Ait-Oukhatar et al., 1999) have demonstrated that Fe bound to the phosphoserine residues of low-molecular-weight CPPs, namely beta-casein, improved their ability to treat anaemia and restore Fe storage tissues, when compared with Fe bound to whole casein and inorganic salts. It has been shown that Ca-binding phosphopeptides have anticariogenic effects via inhibition of caries lesion through recalcification of the dental enamel—hence, their application in the treatment of dental diseases has been proposed (Clare; Swaisgood, 2000; Reynolds, 1987; Tirelli et al., 1997), namely as additives to toothpaste (Reynolds, 1994). The consumption of cheese has an anticariogenic effect as well; a significant correlation was found between the high content of casein in cheese and the caries-protective effect thereof (Pause; Lembke, 1993). Moreover, anticariogenic activity has also been reported for phosphopeptides from eggs (viz. phosvitin and phosphophorin) (Reynolds, 1994; Tirelli et al., 1997).

Glycomacropeptide: The glycomacropeptide (GMP) is formed during theenzymatic cheesemaking process. Rennet or chymosin hydrolyses the peptide bond between residues 105 and 106 of k-casein, and the resulting molecule, GMP, is eluted in the whey. This molecule contains residues 106–169 of k-casein; the C-terminal portion of said molecule is more hydrophilic, as it contains the oligosaccharides that are O-linked to threonine and serine. The large GMP molecule cannot be absorbed as such, so it has to be broken down into smaller peptides before an effect on blood components arises. GMP is unique in its amino-acid composition—it lacks aromatic amino acids and is rich in branched chain ones. Glycomacropeptide is also known to allow absorption of calcium, iron or zinc. Kelleher et al (2003), studied GMP and alpha-lactalbumin supplementation of infant formulae on the nutritional status in infant rhesus monkeys, and found that both increase zinc absorption—which may allow reduction of formula zinc concentrations and promote a plasma amino-acid pattern similar to that of breastfed infant monkeys, respectively.

Future Application of Bioactive Peptides: The occurrence of many biologically active peptides in dietary proteins is now well-established. Numerous scientific, technological and regulatory issues have, however, to be resolved before these substances can be optimally exploited for human nutrition and health. Firstly, there is a need to develop novel technologies, e.g., chromatographic and membrane separation techniques by means of which active peptide fractions can be produced and enriched. (Korhonen, 2002) Secondly, it is important to study the technological properties of the active peptide fractions and to develop model foods which contain these peptides and retain their activity for a certain period. It is recognized that peptides can be more reactive than proteins, due to their lower molecular weight, and the peptides that are 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 (especially heating) on peptide activity and bioavailability should also be investigated (Korhonen et al., 1998). Furthermore, molecular studies are needed to assess the mechanisms by which the bioactive peptides exert their activities. This research area is currently considered as the most challenging one, due to the understanding that most known bioactive peptides are not absorbed from the gastrointestinal tract into the blood circulation. Their effect is, therefore, likely to be mediated directly in the gut lumen or through receptors on the intestinal cell wall. In this respect, the target function of the peptide concerned is of utmost importance. It is anticipated that in the near future such targets shall be the following lifestyle-related disease groups: a) cardiovascular diseases, b) cancers, c) osteoporosis, d) stress and d) obesity. Peptides derived from dietary proteins offer a promising approach to prevent, control and even treat such disease conditions through a regulated diet (Korhonen; Pihlanto, 2003).

CONCLUSION: Bioactive proteins are a part of our daily food intake and their effects on the human body mainly take place in the lumen and mucosa of the digestive tract. Bioactive peptides, which are encrypted in native peptides, are primarily found in fermented foods, especially in fermented dairy products. The quantities in which they are present are highly dependent on the specific effects of the lactic acid bacteria involved, which can result in substantial variations in traditional dairy products, as our studies on

cheese have shown. In addition, bioactive peptides may be formed or degraded in the digestive tract by proteases and peptidases. The issue of whether bioactive peptides or proteins can have an effect outside the intestinal tract is questionable as their absorption is limited or impossible due to the size of their molecules. In recent years, new peptides demonstrating biological activity have steadily been discovered in different foods. Bioactive peptides from milk proteins have been studied most intensively so far. Nowadays, the application of proteolytic enzymes in combination with new technologies such as chromatographic and membrane separation techniques as well as the use of specific cultures allow the large scale production of bioactive peptides from various food proteins. This enables the enrichment of selected foods with bioactive peptides or the development of new functional foods. Although a large number of physiological effects of bioactive peptides have been described in vitro assays, no clinical studies involving humans have been performed yet, with the exception of those on ACE-inhibitory peptides from milk proteins. For this reason, randomized controlled trials are needed in order to evaluate the health potential of bioactive peptides and proteins in the diet. Bioactive peptides are ubiquitous biomolecules widely abundant and easily obtainable from food proteins. There is no limit therefore to the number of peptides that can be obtained from a single food protein. Each of these peptides may present unique structure and biofunctionalities that can be exploited in the pharmaceutical industry. As research continues to uncover technologies and means to overcome challenges to the use of peptide therapeutics,the prospects of food-derived bioactive peptides will likely fuel in the pharmaceutical on exodus from small molecules and biologics to bioactive peptides.

It is assumed that much attention has been paid recently by researchers toward marine compounds as the safe and efficient agents in prevention or treatment of chronic diseases. Consequently, a large number of bioactive agents from marine organisms have been identified based on the specific assay system or screening approach. Interestingly, marine peptides have been found due to their various biological activities and health beneficial effects. Moreover, the extensive studies marine organisms-derived peptides will contribute to the generation of novel functional food as well as pharmaceutical products.

REFERENCES:

1. Abubakar, A.; Saito, T.; Kitazawa, H.; Kawai, Y.; Itoh, T. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. Journal of Dairy Science, v.8, p. 3131–3138, 1998.

2. Adams, M. R.; Golden, D. L.; Anthony, M. S.; Register, T. C.; Williams, J.K. The inhibitory effect of soy protein isolate on atherosclerosis in mice does not require the presence of ldl receptors or alteration of plasma lipoproteins. Journal of Nutrition, 132, p. 43–49, 2002.

3. Adams, M. R.; Golden, D. L.; Franke, A. A.; Potter, S. M.; Smith, H. S.; Anthony, M. S. Dietary soy beta-conglycinin (7S globulin) inhibits atherosclerosis in mice. Journal of Nutrition, v. 134, p. 511–516, 2004.

4. Ait-Oukhatar, N.; Bouhallab, S.; Arhan, P.; Maubois, J. L.; Drosdowsky, M.; Bougle, D. Iron tissue storage and haemoglobin levels of deficient rats repleted with iron bound to the caseinophosphopeptide 1–25 of beta-casein. Journal of Agricultural and Food Chemistry, p.47, n. 7, p.2786–2790, 1999.

5. Anderson, J. W.; Johnstone, B. M.; Cook-Newell, M. E. Meta-analysis of the effects of soy protein intake on serum lipids. New England Journal of Medicine v. 333, p. 276–282, 1995.

6. Armstrong, W. B.; Kennedy, A. R.; Wan, X. S.; Atiba, J.; Mclaren, C. E.; Meyskens, F. L. Single-dose administration of Bowman-Birk inhibitor concentrate in patients with oral leukoplakia. Cancer Epidemiology, Biomarkers and Prevention, 9, 43–47, 2000.

7. Belem, M. A. F.; Gibbs, B. F.; Lee, B. H. Proposing sequences for peptides derived from whey fermentation with potential bioactive sites. Journal of Dairy Science, v. 82, p. 486–93, 1999.

8. Blunden, G. Biologically active compounds from marine organisms. Phytotherapy Research, v. 15, p. 89–94, 2001.

9. Brantl, V.; Teschemacher, H.; Henschen, A.; Lottspeich, F. Novel opioid peptides derived from casein (b-casomorphins) I. Isolation from bovine casein peptone. Hoppe-Seyleros Zeitschrift fur Physiologische und Chemie, v. 360, p. 1211–1216, 1979.

10. Chabance, B.; Marteau, P.; Rambaud, J. C.; Migliore-Samour, D.; Boynard, M.; Perrotin, P.; Guillet, R., Jolles, P.; Fiat, A. M. Casein peptide release and passage to the blood in humans during digestion of milk and yogurt. Biochemie, v. 80, n. 2, p. 155–165, 1998.

11. Christensen, J. E.; Dudley, E. G.; Pederson, J. A.; Steele, J. L. Peptidases and amino acid catabolism in lactic acid bacteria. Antonie van Leeuwenhoekv, 76, p. 217–246, 1999.

12. Clare, D. A.; Catignani, G. L.; Swaisgood, H. E. Biodefense properties of milk: The role of antimicrobial proteins and peptides. Current Pharmaceutical Design, v. 9, n. 16, p. 1239–1255, 2003.

13. Cohen, L. A.; Zhao, Z.; Pittman, B.; Scimeca, J.A. Effect of intact and isoflavone depleted soy protein on NMU induced rat mammary tumorigenesis. Carcinogenesis, v. 21, p. 929–935, 2000.

14. Cohen, L. A.; Zhao, Z.; Pittman, B.; Scimeca, J.A. Effect of intact and isoflavone depleted soy protein on NMU induced rat mammary tumorigenesis. Carcinogenesis, v. 21, n. 5, p. 929–935, 2000.

15. Daemon, M.; Lombardi, D.; Bosman, F.; Schwartz, S. Angiotensin II induces smooth muscle cell proliferation in the normal and injured rat arterial wall. Circulation Research, v. 68, p. 450–456, 1991.

16. Dia, V. P.; Wang, W.; Oh, V. L.; Delumen, B.O. & De Mejia, E.G. Isolation, purification and characterisation of lunasin from defatted soybean flour and in vitro evaluation of its anti-inflammatory activity. Food Chemistry, v. 114, n. 13, p. 108-115, 2009.

17. Erdmann, K.; Cheung, B. W. Y.; Schroder, H. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular diseases. Journal of Nutritional Biochemistry v. 19, n. 10, p. 643–654, 2008.

18. Fiat, A. M.; Levy-Toledano, S.; Caen, J. P.; Jollés, P. Biologically active peptides of casein and lactotransferrin implicated in platelet function. Journal of Dairy Research, v. 56, p. 351-355, 1989.

19. Fitzgerald, R.; Murray, B. A. Bioactive peptides and lactic fermentations. International Journal of Dairy Technology, v. 59, p. 118-125, 2006.

20. Fukui, K.; Tachibana, N.; Wanezaki, S.; Tsuzaki, S.; Takamatsu, K.; Yamamoto, Y.; Hashimoto, Y.; Shimoda,T. Isoflavone-free soy protein prepared by column chromatography reduces plasma cholesterol in rats. Journal of Agricultural Food Chemistry, v. 50, p. 5717–5721, 2002.

21. Gaetke, L. M.; Chow, C. K. Copper toxicity, oxidative stress, and antioxidant nutrients. Toxicology, v. 189, p. 147–163, 2003.

22. Galvez, A. F. Method of large-scale production and method of testing of the biological activity of a substance from soybean. Patent Cooperation Treaty Patent, WO 2001/034808, 2001.

23. Galvez, A. F.; Chen, N.; Macasieb, J.; De Lumen, B.O. Chemopreventive property of a soybean peptide (lunasin) that binds to deacetylated histones and inhibits acetylation. Cancer Resource, 61, p.7473–7478, 2001.

24. Geisterfer, A.; Peach, M.; Owens, G. Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. Circular Resource, 62, p. 749–756, 1988.

25. Gibbs, B. F.; Zougman, A.; Masse, R.; Mulligan, C. Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented foods. Food Resource International, p. 37, n. 2, p. 123–31, 2004

26. Gill, I.; López-Fandiño, R.; Jorba, X.; Vulfson, E.N. Biologically active peptides and enzymatic approaches to their production. Enzyme Microbiology Technology, p.18, n. 3, p. 162–83, 1996.

27. Gobbetti, M.; Minervini, F.; Rizzello, C. G. Angiotensin Iconverting-enzyme-inhibitory and antimicrobial bioactive peptides. International Journal of Dairy Technology, 57, v. 172–188, 2004.

28. Gobbetti, M.; Stepaniak, L.; De Angelis, M.; Corsetti, A.; Di Cagno, R. Latent bioactive peptides in milk proteins: Proteolytic activation and significance in dairy processing. Critical Reviews in Food Science and Nutrition, v. 42, p. 223–239, 2002.

29. Hartmann, R.; Meisel, H. Food-derived peptides with biological activity: from research to food applications. Current Opinion in Biotechnology, p. 18, n. 2, v. p. 163-169, 2007.

30. Hsieh, C. C.; Henandez-Ledesma, B.; Jeong, H. J.; Park, J. H.; De Lumen, B. O. Complementary roles in cancer prevention: protease inhibitor makes the cancer preventive peptide lunasin bioavailable. Public Library of Science One, v. 5, n.1,

31. Hsu, C. S.; Shen, W. W.; Hsueh, Y. M.; Yah, S. L. Soy isoflavone supplementation in postmenopausal women. Effectson plasma lipids, antioxidant enzyme activities and bone density. Journal of Reproduction Medical, 46, v. 221–226, 2001.

32. Iigo, M., Kuhara, Y., Ushida, Y., Sekine, K., Moore, M.A.; Tsuda, H. Milk and dairy products in cancer prevention: focus on bovine lactoferrin. Clinical Experiment Metastasis, v. 17, p. 35–40, 1999.

33. Jakala, P.; Vapaatalo, H. Antihypertensive peptides from milk proteins. Pharmaceuticals v. 3, p. 251-272, 2010.

34. Jeong, H. J.; Jeong, J. B.; Kim, D. S.; De Lumen, B.O. The cancer preventive peptide lunasin from wheat inhibits core histone acetylation.

Journal of Agricultural Food Chemistry, v. 55, p. 632–637, 2007.

35. Jeong, H. J.; Jeong, J.B.; Kim, D. S.; Park, J. H.; Lee, J. B.; Kweon, D. H.; Chung, G. Y.; Seo, E. W.; De Lumen, B. O. The cancer preventive peptide lunasin from wheat inhibits core histone acetylation. Cancer Literature, 255, 42–48, 2007.

36. Jeong, H. J.; Lam, Y.; De Lumen, B. O. Barley lunasin suppresses ras-induced colony formation and inhibits core histone acetylation in mammalian cells. Journal of Agricultural Food Chemistry, v. 50, p. 5903–5908, 2002.

37. Jollés, P.; Caen, J. P. Parallels between milk clotting and blood clotting: opportunities for milk-derived products. Trends in Food Science and Technology, v. 2, p. 42-43, 1991.

38. Jollès, P.; Henschen, A. Comparison between the clotting of blood and milk. Trends in Biochemical Science, v. 7, p. 325-328, 1982.

39. Jolles, P.; Levy-Toledano, S.; Fiat, A. M.; Soria, C.; Gillessen, D.; Thomaidis, A. Analogy between fibrinogen and casein effect of an undecapeptide isolated from kappa-casein on platelet function. European Journal of Biochemistry, v. 158, p. 379-382, 1986.

40. Kannel, W. B.; Castelli, W. P.; Gordon, T.; Mcnamara, P. M. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Annals of Internal Medicine, v. 74, p. 1–12, 1971.

41. Kelleher, S. L.; Chatterton, D.; Nielsen, K.; Lonnerdal, B. Glycomacropeptide and alpha-lactalbumin supplementation of infant formula affects growth and nutritional status in infant rhesus monkeys. The American Journal of Clinical Nutrition, v. 77, p. 1261–1268, 2003.

42. Kim, D. N.; Lee, K. T.; Reiner, J. M.; Thomas, W.A. Increased steroid excretion in swine fed high-fat, high-cholesterol diet with soy protein. Experimental Molecular Pathology, v. 33, p. 25–35, 1980.

43. Kim, S. E.; Kim, H. H.; Kim, J.Y.; Kang, Y. I.; Woo, H. J.; Lee, H. J. Anticancer activity of hydrophobic peptides from soy proteins. BioFactors, v. 12, p. 151–155, 2000.

44. Kitts, D. D.; Weiler, K. A. Bioactive proteins and peptides from food sources applications of bioprocesses used in isolation and recovery. Current Pharmaceutical Design, v. 9, p. 1309-1323, 2003.

45. Kobayashi, H.; Suzuki, M.; Kanayama, N.; Terao, T. Clinical Experimental Metastasis, v. 21, p. 159–166, 2004.

46. Korhonen H, Pihlanto A. Food-derived bioactive peptides—opportunities for designing future foods. Current Pharmaceutical Design v. 9, n. 16, p. 1297–308, 2003.

47. Korhonen, H. *Technology* options for new nutritional concepts. International Journal of Dairy Technology v. 55, p.79-78, 2002.

48. Korhonen, H.; Pihlanto-Leppala, A. Milk protein-derived bioactive peptides-novel opportunities for health promotion. International Dairy Federation Bulletin, 363: 17–26, 2001.

49. Korhonen, H.; Pihlanto-Leppälä, A.; Rantamäki, P.; Tupasela. T. Impact of processing on bioactive proteins and peptides. Trends of Food Science Technology, v. 9, p. 307, 1998.

50. Korhonen, H. J. T.; Pihlanto-Leppala, A. Milk-derived bioactive peptides : formation and prospects for health promotion. In: Edited by Colette Shortt and John O'Brien. Handbook of functional dairy products. Functional foods and neutraceuticals series, p. 109-124, 2004.

51. Leblanc, J. G.; Matar. C.; Valdez, J. C.; Leblanc, J.; Perdigon, G. Immunomodulating effects of peptidic fractions issued from milk fermented with Lactobacillus helveticus. Journal of Dairy Science, v. 85, p. 2733–42, 2002.

52. Lee, T. G.; Maruyama, S. Isolation of HIV-1 protease inhibiting peptides fromthermolysin hydrolysate of oyster proteins. Biochemical and Biophysical Research Communication, v. 253, p. 604–608, 1998.

53. Lefranc, C. Cool, calm and collected. Dairy Industries International, p. 36–37, 2001.

54. Lahov, E.; Regelson, W. Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. Food and Chemical Toxicology, v. 34, P. 131–145, 1996.

55. Maeno, M.; Yamamoto, N.; Takano, T. Identification of an antihypertensive peptide from casein hydrolysate produced by a proteinase from *Lactobacillus helveticus* CP790. Journal of Dairy Science, v. 79, p. 1316-1321, 1996.

56. Marcuse, R. The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. Journal of American Oil Chemistry Society, v. 39, p. 97–103, 1962.

57. Matar, C.; Leblanc, J. G.; Martin, L.; Perdigo, G. Biologically active peptides released in fermented milk: Role and functions. In E. R. Farnworth (Ed.),Handbook of fermented functional foods. Functional foods and nutraceuticals series (pp. 177–201). Florida, USA: CRC Press, 2003.

58. Matsui T., Yukiyoshi A., Doi S., Sugimoto H., Yamada H, Matsumoto K. 2002. Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR. Nutritional Biochemistry, v. 13, n. 2, p. 80–86, 2002.

59. Megias, C.; Pedroche, J.; Yust, M. M.; Giron-Calle, J.; Alaiz, M.; Milan, F.; Vioque, J. Affinity purification of copper-chelating peptides from sunflower protein hydrolysates. Journal of Agricultural Food Chemistry, v. 55, p. 6509–6514, 2007.

60. Meisel, H.; Fitzgerald, R. J. Biofunctional peptides from milkproteins: Mineral binding and cytomodulatory effects. Current Pharmaceutical Design, n. 9, p. 1289–1295, 2003.

61. Meisel, H.; Fitzgerald, R. J. Opioid peptides encrypted in intact milk protein sequences. British Journal of Nutrition, v. 84, p. 27–31, 2000.

62. Mellander, O. The physiological importance of the casein phosphopeptide calcium salts. 11. Peroral calcium dosage of infants. Act of the Society of Medicine of Uppsala, 55, p. 247–255, 1950.

63. Merzametov, M. M.; Gadzhieva, L. I. Certain amino acids as antioxidants in butterfat. Izv. Ucheb. Zaved. Pischew. Technology, v. 115, p. 21–27, 1976.

64. Messina, M.; Flickinger, B. Hypothesized anticancer effects of soy: evidence points to isoflavones as the primary anticarcinogens. Pharmaceutical Biology, v. 4, p. 6–23, 2002.

65. Messina, M.J.; Loprinzi, C.L. Soy for breast cancer survivors: a critical review of the literature. The Journal of Nutrition, v. 131, n. 11, 3095–3108, 2001.

66. Meyskens Jr, F. L. Development of Bowman-Birk Inhibitor for Chemoprevention of Oral Head and Neck Cancer. Annals of New York Academy of Science, 952, p. 116–123, 2001.

67. Migloire-Samour, D.; Floch, F.; Jolles, P. Biologically active casein peptides implicated in immunomodulation. Journal of Dairy Research, v. 56, n. 3, p. 357–362, 1989

68. Mine, Y.; Kovacs-Nolan, J. New insights in biologically active proteins and peptides derived from hen's egg. Worlds Poultry Science Journal, v. 62, p. 87–95, 2006.

69. Moriyama, T.; Kishimoto, K.; Nagai, K.; Urade, R.; Ogawa, T.; Utsumi, S.; Maruyama, N.; Maebuchi, M. Bioscience Biotechnology Biochemistry, 68: 352–359, 2004.

70. Nagaoka, S.; Miwa, K.; Eto, M.; Kuzuya, Y.; Hori, G.; Yamamoto, K. Soy protein peptic hydrolysate with bound phospholipids decreases micellar solubility and cholesterol absorption in rats and caco-2 cells. Journal of Nutrition, v. 129, p. 1725–1730, 1999.

71. Nagpal, R.; Behare, P.; Rana, R.; Kumar, A.; Kumar, M.; Arora, S., Morotta, F.; Jain, S.; Yadav, H. Bioactive peptides derived from milk proteins and their health beneficial potentials: an update. Food & Function, v. 2, n. 1, p. 18-27, 2011

72. Nakamura, Y.; Yamamoto, M.; Sakai, K.; Okubo, A.; Yamazaki, S.; Takano, T. Purification and characterization of angiotensin Iconverting enzyme inhibitors from sour milk. Journal of Dairy Science, v. 78, p. 777–783, 1995.

73. Ndiaye, F.; Vuong , T.; Duarte, J.; Aluko, R. E.; Matar, C. Antioxidant, anti-inflammatory and immunomodulatory properties of an enzymatic protein hydrolysate from yellow field pea seeds. European Journal of Nutrition, v. 51, p. 29-37, 2012

74. Nielsen, M. S.; Martinussen, T.; Flambard, B.; Sorensen, K.; Otte, J. Peptide profiles and angiotensine-I-converting enzyme inhibitory activity of fermented milk products: effect of bacterial strain, fermentation, pH and storage time. International Dairy Journal, v. 19, n. 3, p. 155-165, 2009.

75. Odani, S.; Koide, T.; Ono, T. Amino acid sequence of a soybean (Glycine max) seed polypeptide having a poly (L-aspartic acid) structure. The Journal of Biological Chemistry, v. 262, n. 22, 10502-10505, 1987a.

76. Odani, S.; Koide, T.; Ono, T.; Seto, Y.; Tanaka, T. Soybean hydrophobic protein. Isolation, partial characterization and the complete primary structure. European Journal of Biochemistry, v. 162, n. 3, p. 485-491, 1987b.

77. Okamoto, A.; Hanagata, H.; Matsumoto, E.; Kawamura, Y.; Koizumi, Y.; Yanagida, F. Angiotensin I converting enzyme inhibitory activities of various fermented foods. Bioscience, Biotechnolology and Biochemistry, n. 59, p. 1147-1149, 1995.

78. Papadimitriou, C. G., Vafopoulou-Mastro jiannaki, A.; Silva, S. V.; Gomes, A. M.; Malcata, F. X.; Alichanidis, E. Identification of peptides in traditional and probiotic sheep milk yoghurt with angiotensin I-converting enzyme (ACE)-inhibitory activity. Food Chemistry, v. 105, p. 647–656, 2007.

79. Pause, B.; Lembke, J. Comparative examination of anticaries effect of cheese. II. Results and conclusions. Milchwissenschaft, v. 48, n. 3, p. 137–141, 1993.

80. Pena-Ramos, E. A.; Xiong, Y. L. Antioxidant Activity of Soy Protein Hydrolysates in a Liposomal System. Journal of Food Science, v. 67, p. 2952–2956, 2002.

81. Pihlanto, A. Antioxidative peptides derived from milk proteins. International Dairy Journal, v. 16, 1306-1314, 2006.

82. Pihlanto-Leppala, A. Bioactive peptides derived from bovine whey proteins: opioid and ace-inhibitory peptides. Trends in Food Science and Technology, v. 11, p. 347–356, 2000.

83. Pihlanto-Leppala, A.; Antila, P.; Mantsala, P.; Hellman, J. Opioid peptides produced by in vitro proteolysis of bovine caseins. International Dairy Journal, v. 4, p. 291–301, 1994.

84. Pihlanto-Leppala, A.; Rokka, T.; Korhonen, H. Angiotensin Iconverting enzyme inhibitory peptides derived from bovine milk proteins. International Dairy Journal, v. 8, p. 325–331, 1998.

85. Potter, S. M. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. Journal of Nutrition v. 125, p. 606–611, 1995

86. Pryme, I.F.; Bardocz, S.; Pusztai, A.; Ewen, S.W. Dietary mistletoe lectin supplementation and reduced growth *of* a murine non-Hodgkin lymphoma. Histology and Histopathology v. 17, p. 261–271, 2002

87. Quirós, A.; Hernández-Ledesma, B.; Ramos, M.; Amigo, L.; Recio, I. Angiotensin-converting enzyme inhibitory activity of peptides derived from caprine kefir. Journal of Dairy Science, v. 88, n.10, p. 3480-3487, 2005.

88. Revankar, G.D. *Proline as an antioxidant in fish oil.* Journal of Food Science and Technology Mysore, v. 11, p. 10–11, 1974.

89. Reynolds, E. C.; Riley, P. F.; Adamson, N. J. A selective purification procedure for multiple phosphoseryl-containing peptides and methods for their identification. Analytical Biochemistry, p. 217, n. 2, p. 277–284. 1994.

90. Reynolds, E.C. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. Journal of Dental Research, 66, p. 1120–1127, 1987.

91. Riison, Y.; Sims, R. J.; Fioriti, J.; A. Effect of amino acids on autoxidation of sunflower *oil in emulsions.* Journal of American Oil Chemistry Society, v. 57, p. 354–359, 1980.

92. Rojas-Ronquillo, R.; Cruz-Guerrero, A.; Flores-Najera, A.; Rodriguezserrano, G.; Gomez-Ruiz, L.; Reyes-Grajeda, J. P.; Jimenez-Guzman, J.; Garcia-Garibay, M. Antithrombotic and angiotensin-converting enzyme inhibitory properties of peptides released from bovine casein by *Lactobacillus casei* Shirota. International Dairy Journal, v. 26, p. 147-154, 2012.

93. Rutherfurd-Markwick, K. J.; Moughan, P. J. Bioactive peptides derived from food. Journal of AOAC International, v.88, p. 955–966, 2005.

94. Sagara, M.; Kanda, T.; Njelekera, M.; Teramoto, T.; Armitage, L.; Birt, N.; Birt, C.; Yamori, Y. Effects of dietary intake of soy protein and isoflavones on cardiovascular disease risk factors in high risk, middle-aged men in Scotland. Journal of American College of Nutrition, v. 23, p. 85–91, 2004.

95. Sandre, C.; Gleizes, A.; Forestier, F.; Gorges-Kergot, R.; Chilmonczyk, S.; Leonil, J.; Moreau, M. C.; Labarre, C. A peptide derived from bovineb-casein modulates functional properties of bone marrow-derived macrophages from germfree andhuman flora-associated mice. The Journal of Nutrition, v. 131, p. 2936–2942, 2001.

96. Setchell, K. D. R. Phytoestrogens: the biochemistry, physiology, and implications for human health of soy isoflavones. American Society for Clinical Nutrition, v. 68, n. 6, p. 1333-1346, 1998.

97. Shahidi, F.; Ying Zhong, Y. Bioactive Peptides, Journal of AOAC International, v. 91, n. P.914-931, 2008.

98. Shin, Z. I.; Yu, R.; Park, S. A.; Chung, D. K.; Ahn, C. W.; Nam, H. S.; Kim, K. S.; Lee, H. J. His-His-Leu,an angiotensin I converting enzyme inhibitory peptide derived from Korean soybeanpaste, exerts antihypertensive activity in vivo. Journal of Agricultural Food Chemistry, v. 49, p. 3004–3009, 2001.

99. Silva-S Anchez, C.; Barba De La Rosa, A. P.; Leeon-Galvan, M. F.; De Lumen, B. O.; De Leeon Rodrriguez, A.; Gonzalez De Mejia, E. Bioactive peptides in amaranth (*Amarathus hypochondriacus*) seed. Journal of Agricultural Food Chemistry, v. 56, p. 1233–1240. 2008.

100. Sims, R. J.; Fioriti, J. A. Methional as an antioxidant for vegetable oils. Journal of American Oil Chemistry Society, v. 54, p. 4–7, 1977.

101. Sipola, M.; Finckenberg, P.; Korpela, R.; Vapaatalo, H.; Nurminen, M.L. Effect of long-term intake of milk products on blood pressure in hypertensive rats. Journal of Dairy Research, v. 69, p. 103–111, 2002

102. Sirtori, C. R.; Agradi, E.; Mantero, O.; Conti, F.; Gatti, E. Soybean-protein diet in the treatment of type-II hyperlipoproteinaemia. Lancet, v. 1, p. 275–277, 1977.

103. Sugano, M.; Goto, S.; Yamada, Y.; Yoshida, K.; Hashimoto, Y.; Matsuo, T.; Kimoto, M. Cholesterol-lowering activity of various undigested fractions of soybean protein in rats. Journal of Nutrition, v. 120, p. 977–985, 1990.

104. Tachibana, N.; Matsumoto, I.; Fukui, K.; Arai, S.; Kato, H.; Abe, K.; Takamatsu, K. Intake of soy protein isolate alters hepatic gene expression in rats. Journal of Agricultural Food Chemistry, v. 53, p.4253–4257, 2005.

105. Takahashi, M.; Moriguchi, S.; Yoshikawa, M.; Sasaki R. Isolation and characterization of oryzatensin: a novel bioactive peptide with ileum-contracting and immune-modulating activities derived from rice albumin. Biochemistry and Molecular Biology International, v. 33, p. 1151–8, 1994.

106. Takano, T. Anti-hypertensive activity of fermented dairy products containing biogenic peptides. Antony van leeuwenhoek, v. 82, p. 333-340, 2002.

107. Takano, T. Milk derived peptides and hypertension reduction. International Dairy Journal, v.8, p. 375-381, 1998.

108. Teschemacher, H. Opioid receptor ligands derived from food proteins. Current Pharmaceutical Design, v. 9, p. 1331–1344, 2003.

109. Tirelli, A.; De Noni, I.; Resmini, P. Bioactive peptides in milk products. Italian Journal of Food Science, v. 9, p. 91–98, 1997.

110. Tsai, J. S.; Chen, T. J.; Pan, B. S.; Gong, S. D.; Chung, M. Y. Antihypertensive effect of bioactive peptides produced by protease-facilitated lactic acid fermentation of milk. Food Chemistry, v. 106, p. 552–558, 2008.

111. Tsai, P.; Huang, P. Effects of isoflavones containing soy protein isolate compared with fish protein on serum lipids and susceptibility of low density lipoprotein and liver lipids toin vitro oxidation in hamsters. The Journal of Nutritional Biochemistry, v. 10, n. 11, p. 631–637, 1999.

112. U. S. Food and Drug Administration Federal Register. v. 64, p. 57688–57733, 1999.

113. Walther, B.; Sieber. R. Bioactive proteins and peptides in foods, p. 1-12, 2011.

114. Wang, W.; Bringe. N.A.; Berhow, M.A.; Gonzalez De Mejia, E. β-Conglycinins among sourcesof bioactives in hydrolysates of different soybean varieties that inhibit leukemia cells in vitro. Journal of Agricultural Food Chemistry, v. 56,p. 4012–4020, 2008.

115. Wang, Y.; Jones, P. J.; Ausman, L. M.; Lichtenstein, A. H. Soy protein reduces triglyceride levels and triglyceride fatty acid fractional synthesis rate in hypercholesterolemic subjects. Atherosclerosis, v. 173, p. 269–275, 2004.

116. Williams, A. G.; Noble, J.; Tammam, J.; Lloyd, D.; Banks, J. M. Factors affecting the activity of enzymes involved in peptide and amino acid catabolism in non-starter lactic acid bacteria isolated from Cheddar cheese. International Dairy Journal, v. 12, p. 841–852, 2002.

117. Wu, J.; Ding, X. Hypotensive and physiological effect of angiotensin converting enzyme inhibitory peptides derived from soy protein on spontaneously hypertensive rats. Journal of Agricultural and Food Chemistry, v. 49, p. 501-506, 2001.

118. Xiao, C. W. Health effects of soy protein and isoflavones in humans. The Journal of Nutrition, v.138, n.6, p. 1244-1249, 2008.

119. Yamamoto, M.; Akino, A.; Takano, T. Antihypertensive effect of the peptides derived from casein by an extracellular proteinase from Lactobacillus helveticus CP790. Journal of Dairy Science, v. 77, p. 917-922, 1994.

120. Yamamoto, N.; Ejiri, M.; Mizuno, S. Biogenic peptides and their potential use. Current Pharmaceutical Design v. 9, n. 16, p. 1345–55, 2003.

121. Yang, R.; Zhang, Z.; Pei, X.; Han, X.; Wang, J.; Wang, L.; Long, Z.; Shen, X.; Li, Y. Immunomodulatory effects of marine oligopeptide preparation from Chum Salmon (*Oncorhynchus keta*) in mice. Food Chemistry, v.113, p. 464–70, 2009.

122. Yoo, Y. C.; Watanabe, S.; Watanabe, R.; Hata, K.; Shimazaki, K.; Azuma, I. Bovine lactoferrin and lactoferricin, a peptide derived from bovine lactoferrin, inhibit tumor metastasis in mice. Japanese Journal of Cancer Research, 88, p.184–190, 1997.

123. Yoshikawa, M.; Yamada, Y.; Matoba, N.; Utsumi, S.; Maruyama, N.; Onishi, K. Study on introducing new physiological function into soy protein by genetic engineering. Daizu Tanpakushitsu Kenkyu, v. 5, p. 26-30, 2002.

124. Yamamoto, T.; Shima, T.; Furuita, H.; Suzuki, N. Effect of water temperature and short-term fasting on macronutrient self-selection by Common carp (*Cyprinus carpio*). Aqua-culture, n. 220, p. 655–666, 2003.

125. Zhan, S.; Ho, S. C. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. American Journal of Clinical Nutrition, 81, p. 397–408, 60, 2005.