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REVIEW ARTICLE

Bioactive peptides of animal origin: a review

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Abstract Bioactive peptides are specific protein fragments which, above and beyond their nutritional capabilities, have a positive impact on the body's function or condition which may ultimately influence health. Although, inactive within the sequence of the parent proteins, these peptides can be released during proteolysis or fermentation and play an important role in human health by affecting the digestive, endocrine, cardiovascular, immune and nervous systems. Several peptides that are released in vitro or in vivo from animal proteins have been attributed to different health effects, including antimicrobial properties, blood pressure-lowering (ACE inhibitory) effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, opioid activities, enhancement of mineral absorption and/or bioavailability, cytomodulatory and immunomodulatory effects, antiobesity, and anti-genotoxic activity. Several functional foods based on the bioactivities of these peptides with scientifically evidenced health claims are already on the market or under development by food companies. Consumer's increasing interest in these products has given an impetus to the food industry and scientific sector who are continuously exploring the possibilities for the development of new functional products based on these peptides. In this review, we describe above stated properties of bioactive peptides of animal origin.

Keywords Bioactive peptides · Animal origin · Production · Bioactivities

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Introduction

The importance of proteins in the diet has been increasingly acknowledged over the last two decades as a result of new scientific findings in the field of nutrition. The value of proteins as an essential source of amino acids is well documented, but recently it has been recognized that dietary proteins exert many other functionalities in vivo by means of biologically active peptides. Inactive within the sequence of the parent protein, such peptides can be released by digestive enzymes during gastrointestinal transit or by fermentation or ripening during food processing (Korhonen 2009; Chakrabarti et al. 2014; Dziuba and Dziuba 2014).

Defined as "a food component that can affect biological processes or substrates and, hence, have an impact on body function or condition and ultimately health", a bioactive dietary substance should give a measurable biological effect in the range of doses it is usually assumed in the food and this bioactivity should be measured at a physiologically realistic level (Moller et al. 2008). Following this definition, milk, meat, fish and egg-derived bioactive peptides are their respective components that are able to influence some physiological functions, finally acting on body health condition. Until now numerous bioactive substances have been studied but an increasing interest is focused on bioactive peptides of animal-origin, particularly milk- and egg-derived, because at present, livestock products like bovine milk, egg, cheese and dairy

products seem to be extremely important sources of bioactive peptides derived from food (Urista et al. 2011; Dave et al. 2014).

Mellander in 1950 was the first to report the bioactive peptides when he suggested that caseinophosphopeptides (casein-derived phosphorylated peptides) enhanced vitamin D-independent bone calcification in rachitic infants (Korhonen and Pihlanto 2003a). Since then the knowledge of bioactive peptides has steadily increased particularly during the last 20 years and numerous peptides exhibiting various activities such as opiate, antithrombotic, anti-hypertensive, antioxidative, antibacterial, immunomodulation or as relative to mineral utilization and cholesterol-lowering properties have been reported (Mils et al. 2011; Stuknyte et al. 2011; Suarez-Jimenez et al. 2012; Zambrowicz et al. 2013; Bah et al. 2013; Singh et al. 2014; Dave et al. 2014; Chakrabarti et al. 2014; Dziuba and Dziuba 2014; Li and Yu 2014). The peptides derived from food protein can lower blood pressure, inhibit the activity of proline-specific endopeptidases, stimulate the immune system, act as opioids and opioid antagonists, contract smooth muscles, inhibit blood platelet aggregation, inhibit HIV proteinase and oxidation processes, demonstrate antibacterial and fungicidal activity and surface activity, bind ions, participate in mineral transport, determine sensory properties, improve the nutritional value of foods and control body weight (Li and Yu 2014; Dziuba and Dziuba 2014; Chakrabarti et al. 2014). Many peptides are known to reveal multifunctional properties i.e., specific peptide sequences may initiate two or more different biological activities (Meisel and FitzGerald 2003). The activity of peptides is based on the inherent amino acid composition and sequence and become active only when they are released from the precursor protein where they are encrypted. Bioactive peptides usually contain 2–20 amino acid residues although; some have been reported to be more than 20 amino acid residues. Lunasin, for example, is a food-derived peptide with anticancer activity, composed of 43 amino acids.

Once liberated as independent entities, bioactive peptides act as potential metabolism modulators and regulatory compounds with hormone-like activities (Korhonen and Pihlanto 2003a). Upon oral administration, bioactive peptides, may affect the major body systems and have a positive impact on body functions or conditions and may ultimately influence health (Kitts and Weiler 2003). This potential of distinct dietary peptide sequences to promote human health by reducing the risk of chronic diseases or boosting natural immune protection has aroused a lot of scientific interest over the past few years. The application of peptides for therapeutic purposes especially in the field of the treatment of cancer, infections, immunological system disorders and cardiovascular disorders is the focus of interest of many research groups although, these are also recommended as functional food components, i.e., food with designed properties. Due to their physiological and physicochemical versatility, milk, meat and egg-borne bioactive peptides are regarded as highly prominent ingredients for health promoting functional foods or pharmaceutical preparations.

At present, milk proteins are considered the most important source of bioactive peptides. The production and properties of milk protein-derived bioactive peptides have been reviewed in many articles (Clare and Swaisgood 2000; Korhonen and Pihlanto 2003a, b, 2006, 2007; Meisel 2005; Korhonen 2009; Silva and Malcata 2005). Milk-derived bioactive peptides are considered as prominent candidates for various health-promoting functional foods targeted at heart, bone and digestive system health as well as improving immune defence, mood and stress control. Recent studies suggest that bioactive milk peptides may also be beneficial in reducing the risk of obesity and development of type-2 diabetes (Zimecki and Kruzel 2007; Erdmann et al. 2008; Haque and Chand 2008; Moller et al. 2008). Technologies for industrial scale production of such peptides have recently been developed and already there are a few products supplemented with peptides with specific bioactivities on international markets (Korhonen 2009).

Suitability of bioactive peptides as pharmaceutical ingredients

By controlling, directing and/or coordinating inter- and intracellular communications and cellular functions, proteins and peptides play important roles in living body systems (Danquah and Agyei 2012). Peptides with low molecular weight are more bioavailable than proteins or free amino acids from nutritional point of view (Hajirostamloo 2010) and also have been known to be less allergenic than their native proteins which justifies their wide use in the preparation of hypoallergenic infant food formulations (Host and Halken 2004; Danquah and Agyei 2012). Additionally, as natures tool kit, the diverse physiological roles of peptides make them suitable candidates for the development of therapeutic agents (Lax 2010; Agyei and Danquah 2011; Danquah and Agyei 2012). Therefore, in the light of the sizable side effects of synthetic drugs and with the heightened attention to fresher and ‘greener’ foods and nutraceuticals possessing health-preventing or health-promoting properties, the bioactive peptides seems to be the most suitable candidates in the new era of pharmaceutical products (Danquah and Agyei 2012).

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, (c) through the action of enzymes derived from proteolytic microorganisms. Once the structure of bioactive peptides is known, it is also possible to synthesize peptides. Three main approaches are available at present: (1) chemical synthesis; (2) recombinant DNA technology; and (3) enzymatic synthesis (Korhonen and Pihlanto 2003a).

Enzymatic hydrolysis

Enzymatic hydrolysis of whole protein molecules is the most common way to produce bioactive peptides and many of the known bioactive peptides have been produced using digestive enzymes and different enzyme combinations of proteinases like pepsin, trypsin, alcalase, chymotrypsin, pancreatin, pepsin and thermolysin. A large number of studies have demonstrated that biologically active peptides can be produced by hydrolysis of milk proteins by digestive enzymes (Korhonen and Pihlanto 2006; Korhonen 2009). Pepsin, trypsin and chymotrypsin being the most prominent enzymes that have been shown to release a number of antihypertensive peptides, calcium-binding phosphopeptides (CPPs), antibacterial, immunomodulatory and opioid peptides both from different casein (α-, β- and j-casein) and whey proteins, e.g., α-lactalbumin (α-la), β-lactoglobulin (β-lg) and glycomacropeptide (GMP) (Meisel and FitzGerald 2003; Yamamoto et al. 2003; FitzGerald et al. 2004; Gobbetti et al. 2004, 2007; Korhonen 2009). Peptides which inhibit the angiotensin converting enzyme I (ACE) and thereby reduce blood pressure are most studied (Korhonen 2009; Li et al. 2004; Meisel et al. 2006; Murray and FitzGerald 2007; Saito 2008). In some studies (Otte et al. 2007) casein hydrolysates have produced higher ACE-inhibitory activity than whey protein hydrolysates however, tryptic digest of β-lg yielded peptides such as Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) that have been identified with strong antihypertensive activity (Mullally et al. 1997; Maes et al. 2004; Ferreira et al. 2007; Korhonen 2009). For the chemical characterisation and identification of many known bioactive peptides, pancreatic enzymes (preferably trypsin) have been widely employed. Enzymatic hydrolysis by trypsin most commonly produced Angiotensin-converting enzyme (ACE)-inhibitory peptides and calcium-binding phosphopeptides (CPPs) (FitzGerald et al. 2004; Gobbetti et al. 2004; Vermeirssen et al. 2004). Casein micelles successively digested with pepsin and trypsin have given higher yields of CPPs and, in particular, higher amounts of α_{s1}-casein f(59–79) in the hydrolysate than from acid-precipitated casein and casein micelles by tryptic digestion alone (Ono et al. 1998). Moreover, ACE-inhibitory peptides have recently been identified in the tryptic hydrolysates of bovine α_{s2}-casein (Tauzin et al. 2002) and in bovine, ovine and caprine κ-casein macropeptides (Manso and López-Fandino 2003). Alcalase, Thermolysin and Subtilisin are examples of other proteolytic enzymes that have been employed to release various bioactive peptides, including CCPs (McDonagh and FitzGerald 1998; Korhonen 2009), ACE inhibitory (Pihlanto-Leppälä et al. 2000; Vermeirssen et al. 2004; Roufik et al. 2006; deCosta et al. 2007; Korhonen 2009), antibacterial (López-Expósito and Recio 2006; López-Expósito et al. 2007), antioxidative (Pihlanto 2006), immunomodulatory (Gauthier et al. 2006) and opioid-like (Teschemacher 2003; Korhonen 2009). Besides the milk proteins, peptides have been successfully derived from other proteins by thermolysin and trypsin. For example, several hypotensive peptides have been identified from porcine skeletal muscle and corn protein after digestion with thermolysin (Arihara et al. 2001; Nakashima et al. 2002; Murakami and Hirata 2000; Korhonen and Pihlanto 2003a).

Conventional batch hydrolysis or continuous hydrolysis using ultrafiltration membranes are the two methods by which hydrolysis can be performed. Several studies (Mannheim and Cheryan 1990; Chiang et al 1995; Korhonen and Pihlanto 2003a) have indicated several disadvantages of the traditional batch method, such as the relatively high cost of the enzymes and their inefficiency compared to a continuous process. An attractive configuration for this purpose is the enzymatic membrane reactor, which integrates enzymatic hydrolysis, product separation and catalyst recovery into a single operation and has already been widely applied to total conversion of food proteins of various origins, in order to produce hydrolysates with improved functional and/or nutritional properties (Perea and Ugalde 1996; Martin-Orue et al. 1999). Ultrafiltration membrane reactors have been shown to have certain advantages like improved efficiency of enzyme-catalysed bioconversion, increased product yields, be easily scaled up and yielding a consistently uniform product with desired molecular mass characteristics (Mannheim and Cheryan 1990). To separate small peptides from high molecular mass residues and remaining enzymes, ultrafiltration steps using low molecular mass cut-off membranes may be useful. Degradation of bovine β-casein by plasmin in a membrane recycle reactor for the continuous production and isolation of peptide fractions was studied by Visser et al. (1989). A two-step ultrafiltration process was used by Turgeon and Gauthier (1990) to produce a mixture of polypeptides and a fraction rich in small peptides, with molecular mass below 2000 Da (Korhonen and Pihlanto 2003a).

Microbial fermentation

Because of the highly proteolytic nature of many dairy starter cultures, formation of bioactive peptides can be expected during the manufacture of fermented dairy products. Different bioactive peptides released from milk proteins through microbial proteolysis are now very well documented (Matar et al. 2003; FitzGerald and Murray 2006; Gobbetti et al. 2007; Korhonen 2009). ACE-inhibitory peptides have been successfully generated using microbial enzymes (Yamamoto et al. 1994; Maeno

et al. 1996; Korhonen and Pihlanto 2003a). Lactobacillus helveticus strains have been particularly associated with production of antihypertensive peptides by many researchers, the best known of which are ACE-inhibitory tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP). Several rat model and human studies have demonstrated antihypertensive capacity of these peptides (Hata et al. 1996; Masuda et al. 1996; Nakamura et al. 1995a, b; Sipola et al. 2002; Seppo et al. 2003; Mizushima et al. 2004; Aihara et al. 2005; Jauhiainen et al. 2005; Hirota et al. 2007; Korhonen 2009). Production of different bioactive peptides in milk during fermentation by yoghurt bacteria, cheese starter bacteria and commercial probiotic bacteria have also been demonstrated (Gomez-Ruiz et al. 2002; Fuglsang et al. 2003; Gobbetti et al. 2004; Donkor et al. 2007; Korhonen 2009). Chen et al. (2007) reported that the fermentation of milk with a commercial starter culture (mixture of five lactic acid bacteria (LAB) strains) followed by hydrolysis with a microbial protease increased ACE inhibitory activity of the hydrolysate. Antihypertensive effect of the hydrolysate containing two strong ACE-inhibitory tripeptides (Gly-Thr-Trp and Gly-Val-Trp) was demonstrated in an animal model study using spontaneously hypertensive rats (SHR). Several novel ACE-inhibitory peptides in milk fermented with *Enterococcus faecalis* strains isolated from raw milk were identified by Quiros et al. (2007).

Meat has also been used as a valuable protein source for the production of bioactive peptides. Till date, many bioactive peptides have been reported from meat proteins during the process of curing as well as during fermentation. Particularly, the use of meat proteins for the production of ACE inhibitory bioactive peptides is very common. The angiotensin converting enzyme inhibitory peptides generated during the curing of meat products have been studied extensively. For example, dipeptidyl peptidases (DPP) generated antihypertensive peptides among which dipeptide Arg–Pro showed the strongest angiotensin converting enzyme inhibitory activity (Jang and Lee 2005). Utilizing such components to develop novel meat products is extensively under study.

Arihara (2004) evaluated eight different enzymatic hydrolyzates (by using exogenous enzymes) of porcine skeletal muscle proteins for the ACE inhibitoty activity and found that the thermolysin digest had the most potent inhibitory activity among them. Two ACE inhibitory peptides identified were Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro, and were corresponded to the sequence of myosin heavy chain. In addition, these peptides showed significant blood pressure-reducing effect in spontaneous hypertensive rats (Nakashima et al. 2002). In order to produce ACE inhibitory peptides, Saiga et al. (2003) treated chicken breast meat extract with an *Aspergillus* protease and gastric proteases (trypsin, chymotrypsin, and intestinal juice). They observed ACE inhibitory effect in both the extract and hydrolysate of the extract. Three ACE inhibitory peptides having common sequence of Gly-X-X-Gly-X-X-Gly-X-X were identified and the strongest ACE inhibitory activity was observed with Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe peptide. They also evaluated the *Aspergillus* protease hydrolsate of chicken collagen for ACE inhibitory activity and reported that the responsible peptide have the sequence of Gly-Ala-Hyp-Gly-Leu-Hyp-Gly-Pro. Administration of the responsible peptide-containing fraction of hydrolysate in spontaneous hypertensive rats also showed significant reduction in the blood pressure. Hydrolysates of chicken leg bones were evaluated for ACE inhibitory activity by Fu-Yuan et al. (2008). The hydrolysate obtained by Alkalase enzyme showed the highest activity. A peptide with Val-Leu-Ala-Gln-Tyr-Lys sequence from hydrolysates of sarcoplasmic protein extracts of beef was reported to have a very strong ACE inhibitory ability by Jang and Lee (2005). Kazunori et al. (2003) evaluated the pepsin hydrolysate of porcine skeletal troponin C for the ACE inhibitory activity and found that a peptide with RMLGQTPT amino acid sequence had a very high ACE inhibitory activity. Two peptides with amino acid sequence of Gly-Pro-Leu and Gly-Pro-Val with high ACE inhibitory activity were isolated from bovine skin gelatin sequentially digested with Alcalase, Pronase E and collagenase (Kim et al. 2001).

Strong antioxidant activity against lipid oxidation was observed by Sakanaka et al. (2005) who evaluated ground beef homogenates incorporated with casein calcium peptides obtained by using microbial enzyme hydrolysis. Wang and Xiong (2008) investigated the effect of hydrolyzed potato proteins on the oxidation of isolated myofibril proteins in induced (iron-catalyzed and metmyoglobin) oxidizing systems and found that the hydrolyzed potato proteins reduced the oxidation of myofibril proteins in all physicochemical conditions tested. Casein peptides produced using flavourzyme were reported to have greater antioxidant capacity than alcalse-derived ones by Rossini et al. (2009). Those peptides were effective in inhibiting lipid peroxidation of ground beef homogenates and mechanically deboned poultry meat. Zhang and Zhou (2010) incorporated three fractions of soy bean hydrolysates obtained from neutral protease treatment into ground beef and observed significant reduction in lipid peroxidation. These findings indicate the potential of these bioactive peptides in the development of functional meat products. The use and application of artificial antioxidants have become challenging due to potential health hazards related to synthetic antioxidants (Becker 1993; Mendis et al. 2005). Therefore, use of bioactive antioxidant peptides in the development of meat products avoids the potential health risk associated with artificial antioxidants.

Peptide synthesis

Synthesis is the most popular method of obtaining bioactive peptides in a laboratory (Narai-Kanayama et al. 2010; Dziuba

and Dziuba 2014). The length and quantity of the desired peptide are the two criteria that mainly determine the most suitable method for peptide synthesis. The total enzymatic synthesis is currently limited to relatively short sequences. For relatively large peptides recombinant DNA technology is the preferred choice and is suited to products consisting of up to several hundred amino acids. On the laboratory scale, the most widely used current approach to the synthesis of peptides is the chemical one. Two variants of this methodology exist, namely liquid-phase and solid-phase synthesis. Solid-phase approach is the most powerful method for synthesis of peptides composed of about 10 to over 100 residues on a small scale and also for the rapid production of peptide libraries for screening purposes (Gill et al. 1996; Korhonen and Pihlanto 2003a).

The application of recombinant DNA technology produces the product in large quantities from very inexpensive starting materials via fermentation however; it typically requires a long and expensive research and development phase. Attempts to extend this approach to the preparation of short peptides have not yet been truly successful. Both human as well as bovine β-Casomorphins have been produced by genetic engineering techniques. The genes were cloned into a plasmid and transformed to *E. coli* and the fusion with a host protein was used to protect peptides from proteolytic degradation. The required peptides were cleaved from the fusion protein by enzymatic or chemical methods in the final step and the concentration of β-casomorphin in the culture medium was estimated to be 38.22 nmol/l (Baldauf et al. 1994; Meister et al. 1994). Furthermore, genetic engineering can be used to produce enzymes having specific activity in such a manner that they are able to release the desired peptides from the precursor proteins. Despite significant advances, the synthesis of short sequences using genetic engineering methods often remains impractical due to the low expression efficiencies obtained and difficulties encountered in product extraction and recovery (Korhonen and Pihlanto 2003a).

Functional properties of bioactive peptides

Table 1 shows some bioactive peptides of animal origin with different bioactivities whereas Table 2 shows biological activities of egg white proteins. The different bioactivities of bioactive peptides of animal origin are as follows:

Table 1 Bioactive peptides of animal origin with different activities

Body systems	Bioactivity	Peptide/protein	Reference
Cardio vascular system	Antihypertensive	Val-Pro-Pro, Ile-Pro-Pro (milk)	Bu¨ tikofer et al. (2007)
		LKP, IKP, LRP (sardine, bonito, tuna)	Nagai et al. (2006)
		KVREGTTY (egg)	Lee et al. (2006)
		FRADHPPL (egg)	Miguel and Aleixandre (2006)
	Antioxidative	Met-His-Ile-Arg-Leu Tyr-Val-Glu-Glu-Leu (α-LA and β-LG)	Ebringer et al. (2008)
		MHIRL, YVEEL, WYSLAMAASDI (milk)	Hernandez-Ledesma et al.(2005)
		Val-Lys-Ala-Gly-Phe-Ala-Trp-Thr-Ala-Asn-Glu-Glu-Leu-Ser (tuna)	Je et al. (2007)
	Antithrombotic	κ-caseinoglycopeptide (106–171)	Qian et al. (1995)
		κ -casein f106-f112 and f113-f116	Fiat et al. (1989)
	Hypocholesterolemic	Ile-Ile-Ala-Glu-Lys (β-LG)	Ebringer et al. (2008)
Immune system	Antimicrobial	α_s-casein f1-f23	Lahov and Regelson (1996)
	Immunomodulatory	β-casein f191-f193, β-casein f63-f68	Fiat et al. (1989)
	Cytomodulatory	α-Casomorphin (HIQKED(V)) β-casomorphin-7 (YPFPGPI) (milk)	Kampa et al. (1997)
Nervous system	Opioid agonist	α_{s1}-casein f90-f96	Loukas et al. (1983)
	Opioid antagonist	k-casein f33-f38	Chiba et al. (1989)
Gastrointestinal system	Mineral binding	-Ser(P)-Ser(P)-Ser(P)-Glu(E)-Glu(E)-	Sharma et al. (2011)
		VSGVEDVN	Lee and Song (2009a)
		DLGEQYFKG	Lee and Song (2009b)
	Anti-appetizing	Total whey protein	Zhang and Beynen (1993)
	Antimicrobial	Caprine α_{s1} - CN f(24–30) (cheese)	Rizzello et al. (2005)

Table 2 Biological activities of egg white proteins

Egg white proteins	Relative % (*w*/*w*)	Biological activity	Reference
Ovalbumin	54.0	Antibacterial activity	Pellegrini et al. (2004)
		Antihypertensive activity of ovalbumin-derived peptides	Matoba et al. (2001), Yamada et al. (2002), Miguel et al. (2004), Matoba, et al. (1999)
		Immunomodulating activity	Vidovic et al. (2002), Goldberg et al. (2003), He et al. (2003)
Ovotransferrin	12.0	Antimicrobial activity	Aguilera et al. (2003), Baron et al. (2000) Giansanti et al. (2002)
		Antibacterial activity of ovotransferrin Peptide (otap-92)	Ibrahim et al. (2000)
		Immunomodulating activity	Otani and Odashima (1997), Xie et al. (2002)
Ovomucoid	11.0	Ovomucoid serine protease inhibitor	Kato et al. (1987), Hilpert et al. (2003)
		Immunomodulating activity	Holen et al. (2001)
Ovomucin	3.5	Antimicrobial activity	Watanabe et al. (1998), Tsuge et al. (1997a)
		Antiadhesive properties	Kobayashi et al. (2004)
		Antitumor activity	Watanabe et al. (1998), Oguro et al. (2001)
Lysozyme	3.4	Antibacterial activity	Ibrahim et al. (2002), Ibrahim et al. (2001)
		Antiviral activity	Sava (1996), Tenuovo et al. (2002)
		Immunomodulating and Immunostimulating activity	Li-Chan and Nakai (1989), Sava (1996), Sugahara et al. (2000)
		Antitumor activity	Shcherbakova et al. (2002), Pacor et al. (1999)
		Antiviral activity	Ebina et al. (1991)
		Antimicrobial activity	Miyagawa et al. 1994, Maruo et al. 1998
		Antimicrobial activity	Naka (2000), Korant et al. (1986)
		Antitumor activity	Saleh et al. (2003), Premzl et al. (2001)
		Immunomodulating activity	Kato et al. (2000), Verdot et al. (1999)
Avidin	0.05	Antibacterial activity	Korpela et al. (1984)

Adapted from Li-Chan et al. (1995) and Kovacs-Nolan et al. (2005)

Antihypertensive activity

Among the various bioactive peptides, antihypertensive peptides probably seem to be the most studied peptides from exogenous sources such as food. These food-derived antihypertensive peptides have not only been well researched but have also been put to practical use as functional and designer foods. Since hypertension has become a serious health problem, especially in developed countries, and has been considered a risk factor for developing cardiovascular diseases, there has been a growing interest in antihypertensive peptides for their effectiveness in lowering blood pressure. These peptides have been found effective in preventing or treating hypertension mainly by inhibiting the angiotensin-converting enzyme (ACE), which plays a key role in the regulation of blood pressure and electrolyte homeostasis. IPP and VPP peptides are generally described and analysed as inhibitors of the angiotensin I-converting enzyme (Panchaud et al. 2012, Dziuba and Dziuba 2014). Angiotensin I-converting enzyme (ACE, peptidyldi-peptide hydrolase, EC 3.4.15.1) is a key enzyme in the rennin-angiotensin system. This enzyme regulates extracellular fluid volume and arterial vasoconstriction either by converting angiotensin I to the vasoconstrictor angiotensin II or by inactivating the bradykinin (a vasodilatory peptide) and enkephalins (Petrillo and Ondetti 1982). Inhibition of ACE therefore results in a decrease in blood pressure, helping to control hypertension.

The first exogenous ACE inhibitors having an antihypertensive effect in vivo were first discovered in snake venom (Ondetti et al. 1977). Isolated from the enzymatic digest of various food proteins, these ACE-inhibitory peptides are recently the most greatly investigated group of bioactive peptides (Korhonen and Pihlanto 2007). Natural ACE-inhibitory peptides from various sources have been studied including a range of different food proteins from both animal and plant sources and their antihypertensive effect has been reported in hypertensive animal models and human subjects as well. Some general features on the structure-activity relationship

of ACE inhibitory peptides have been described (Meisel 1997a, b; FitzGerald et al. 2004). ACE appears to prefer substrates or competitive inhibitors containing hydrophobic (aromatic or branched side chains) amino acid residues at each of the three C-terminal positions, and it is known that the presence of Pro as a C-terminal or antepenultimate residue enhances binding. On the other hand, ACE only binds weakly to competitive peptide inhibitors that have penultimate Pro residues. In addition, the presence of the positive charge of Lys (ε-amino group) or Arg (guanidino group) as the C-terminal residue may contribute to the inhibitory potency (López-Fandino et al. 2007). In order to exhibit the physiological effects and to get to the peripheral organs, ACE-inhibitory peptides depend on their ability to reach their target sites intact, which may involve survival of gastrointestinal digestion and absorption through the intestinal epithelium (Vermeirssen et al. 2004). Several in vitro studies have demonstrated that release of ACE inhibitory peptides upon digestion of food proteins and resistance of ACE-inhibitory sequences to gastrointestinal digestion are essential factors in determining ACE-inhibitory activity of the peptides (Vermeirssen et al. 2003; Gómez-Ruiz et al. 2004). Action of brush-border peptidases, the recognition by intestinal peptide transporters, and the subsequent susceptibility to plasma peptidases are some other factors that influence the physiological effects of the peptides (Pihlanto-Leppälä 2001). Although, most of the antihypertensive peptides have been found effective in preventing or treating hypertension mainly by inhibiting the angiotensin-converting enzyme, however, antihypertensive peptides with insignificant ACE-inhibitory activity have also been isolated from milk products (Korpela et al. 2000; Maeno et al. 1996; Yamamoto et al. 1999). Antihypertensive peptides can also affect blood pressure by mechanisms other than ACE inhibition. Studies suggest the antihypertensive properties for many bioactive peptides with additional mechanisms to lower blood pressure, such as opioid-like activities and mineral-binding and antithrombotic properties.

Opioids are present in the central nervous system and in peripheral tissues; where they are involved, e.g., in the regulation of circulation (Stefano et al. 1995) and also affect blood pressure (Czapla et al. 1998). Several peptide fragments from casein and whey proteins have been found to exhibit opioid-like activity. The first characterized opioid milk peptide agonist was derived from β-casein (β-casomorphin). α-exorphins are the peptides with opioid-like activity derived from α-casein and those derived from κ-casein are called casoxins. α-Lactorphin, derived from α-lactalbumin, has been shown to lower blood pressure in spontaneously hypertensive rats. Because the antihypertensive effect of α-lactorphin was completely prevented by an opioid receptor antagonist naloxone, it has been proposed that the antihypertensive effect is mediated via opioid receptors (Nurminen et al. 2000).

Some peptides have also been shown to increase the solubility of calcium and enhance the absorption of calcium, such as caseinophosphopeptides, (Berrocal et al. 1989; Gagnaire et al. 1996), and some milk peptides have antithrombotic effects by, e.g., inhibiting the aggregation of ADP-activated platelets (Jollès et al. 1986). This might also have some role in the beneficial cardiovascular effects of milk-derived peptides (Jauhiainen and Korpela 2007).

Cholesterol-lowering effect

Conditions like hyperlipidemia, especially hypercholesterolemia, is one of the most important risk factors contributing to the development of cardiovascular diseases. In search of the treatment and prevention of hypercholesterolemia, numerous synthetic drugs and natural extracts with cholesterol-lowering effect have been explored for their potential. Many proteins and their peptides are known to exert a cholesterol-lowering effect (e.g., soy protein, soy 7S globulin, soy protein hydrolysate, enterostatin, soy glycinin fragment, milk β-lactoglobulin hydrolysate, pork protein hydrolysate), among which soybean is the most well recognized source of hypocholesterolemic proteins and peptides. Milk is another important source of bioactive peptides with cholesterol-lowering effect. The cholesterol lowering effect of soybean protein seems to correlate with the bile-acid-binding capacity of these proteins whereas whey proteins affect the cholesterol absorption and the serum cholesterol level by influencing intestinal emulsification and the nature of the resulting micelles. Tryptic hydrolysate of β-lactoglobulin produced a novel hypocholesterolemic peptide (Ile-Ile-Ala-Glu-Lys) (Nagaoka et al. 2001) that was shown to suppress cholesterol absorption by Caco-2 cells in vitro and elicit hypocholesterolemic activity in vivo in rats after oral administration of the peptide solution. Four bioactive peptides corresponding to β-lactoglobulin f9–14, f41–60, f71–75 and f142–146 were identified in the hydrolysate.

Antioxidant activity

Oxidation in the body and in food stuffs has a very important role to play and has been widely recognized. One of the side effects of oxidative metabolism, being essential for the survival of cells, is the production of free radicals and other reactive oxygen species that cause oxidative changes. When an excess of free radicals is formed, they can overwhelm protective enzymes like superoxide dismutase, catalase and peroxidase which cause destructive and lethal cellular effects, like apoptosis, by oxidizing cellular proteins, membrane lipids, DNA, and enzymes thus shutting down cellular process (Sharma et al. 2011).

Proteins, protein hydrolysates, individual peptides, and amino acids have been shown to have significant antioxidant activities. Antioxidative bioactive peptides have been derived

from many hydrolyzed food proteins such as caseins, whey proteins, egg-yolk protein, porcine myofibrillar proteins and aquatic by-product proteins (Pihlanto 2006). They are effective against enzymatic and non-enzymatic peroxidation of lipids and essential fatty acids as free radical scavengers and metal ion chelators. Caseins during hydrolysis by proteolytic enzymes can release antioxidative peptides (Korhonen and Pihlanto 2003a). Peptides derived from α_s-casein have been shown to have free radical-scavenging activity and inhibit enzymatic and non-enzymatic lipid peroxidation (Suetsuna et al. 2000; Rival et al. 2001a, b). Besides being important for the survival of cells in an organism, inhibition of oxidative processes is of particular importance for the food quality. The formation of free radicals results in a deterioration of food quality, for example rancid flavour, unacceptable taste, and reduction of shelf life, while the consumption of foods containing lipid oxidation products has been linked to various diseases, including cancers, diabetes and cardiovascular disease (Ryan et al. 2011).

Several antioxidant peptides have been reported to be generated from meat proteins by enzymatic digestion. Peptides derived from porcine myofibrillar proteins using the proteases Papain and Actinase E represent the first report of antioxidant peptides from the myofibrillar proteins of edible meat. Following digestion, these crude hydrolysates inhibited peroxidation of linoleic acid, DPPH scavenging and metal chelating activities (Ryan et al. 2011). Carnosine and anserine are the two endogenous antioxidative dipeptides found in skeletal muscle (Lynch and Kerry 2000). These peptides have been reported to play many physiological roles, such as prevention of oxidative stress related diseases, and are known to be the most abundant antioxidants in meats (Hipkiss and Brownson 2000).

Many bioactive peptides exhibiting antioxidant properties have been identified from marine organisms like oyster, shrimp, squid, bluemussel, and a variety of fish species. Puffer fish hydrolysate produced strong antioxidant action compared to many other fish sources (Harada et al. 2010). Girgih et al. (2013) reported the antioxidant property of salmon protein hydrolysate as both protein hydrolysate and peptide fractions inhibited the oxidation of linoleic acid. Flounder fish muscle hydrolyzed with α-chymotrypsin has also been reported to possess strong antioxidant activities (Ko et al. 2013). Hydrolysis of Blue mussel (*Mytilusedulis*) protein by the enzyme neutrase is another source for the production of antioxidant peptides. Purification of this hydrolysate revealed the active peptide with the sequence of YPPAK with enhanced hydroxyl and superoxide anion radical scavenging activities (Wang et al. 2013).

Cytomodulatory and anticancer activity

Proteins, peptides, and amino acids have been implicated in preventing the development of different types of cancer. Dairy milk proteins and their peptide derivatives play a role in cancer prevention. CPP has also demonstrated anticarcinogenic activity (Saïd and Dominique 2011). 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 anticancer effect in in vivo studies (Yu et al. 2014; Stiuso et al. 2013). By acting as specific signals that may trigger viability of cancer cells, there is increased evidence that milk-derived peptides may possess cytomodulatory activities (Gobbetti et al. 2007). Bioactive peptides with cytomodulatory activities have been found during bacterial hydrolysis of casein by commercial yogurt starter cultures that affected colon cell Caco-2 kinetics in vitro (McDonald et al. 1994). Bioactive peptides with antiproliferative activity towards leukemia cells have been found during digestion of bovine skimmed milk with cell-free extract of the yeast *Saccharomyces cerevisiae* (Roy et al. 1999). Modulation of cell viability such as proliferation and apoptosis in different human cell culture models has been shown by many purified peptides equivalent to sequences of casein (Hartmann et al. 2000). Meisel and FitzGerald (2003) reported that cytomodulatory peptides derived from casein fractions inhibit cancer cell growth or stimulate the activity of immunocompetent cells and neonatal intestinal cells. The fragments 1–18 and 105–117 from β-casein have been shown to influence the viability as well as the proliferation, differentiation, and apoptosis of different cell types (Phelan et al. 2009).

Jang et al. (2008) investigated the cytotoxic effect of four AMPs from a bovine meat source using the cell lines breast adenocarcinoma (MCF-7), stomach adenocarcinoma (AGS) and lung carcinoma (A549) cells. The peptide GFHI possessed the strongest cytotoxic effect on MCF-7 cells and also decreased the cell viability of AGS cells, while the peptide GLSDGEWQ strongly inhibited the proliferation of AGS cells. Hsu et al. (2010) examined the hydrolysate of tuna dark muscle by-product for potential antiproliferative activity by exposure to the human breast cancer cell line MCF-7. Peptide fractions within the molecular weight range of 400 and 1400 Da exhibited the strongest antiproliferative activity. In these fractions two antiproliferative peptides were identified, i.e., LPHVLTPEAGAT from papain hydrolysate and PTAEGVYMVT from Protease XXIII.

Su et al. (2014) identified a novel anti-cancer bioactive peptide (ACBP), a peptide induced in goat spleen or liver following immunization with human gastric cancer protein extract, which exhibited antitumor activity without measurable side effects. Su et al. (2010) extracted anticancer bioactive peptide (ACBP) from goat spleens with immunization by human gastric cancer extracts and reported that ACBP significantly inhibited the growth of human gastric cancer line BGC-823 in vitro in a dose-dependent manner. In vivo, ACBP dramatically inhibited human gastric tumor growth in a *xenograft* model with no

apparent cytotoxicity to host. The study suggested that ACBP could be a powerful anticancer biological product through induction of cell apoptosis and cell cycle arrest.

Yu et al. (2014) analyzed bioactive peptide-3 (ACBP-3), a novel antitumor agent isolated from goat liver, for the antitumor effect on gastric cancer stem cells (GCSCs) in vitro and in vivo. ACBP-3 dose-dependently decreased the percentage of CD44 (+) cells, suppressing the proliferation of the SC (spheroid colonies) cells and inhibited their clone-forming capacity. Tumor formation from inoculated SC cells took substantially longer when the cells were treated with ACBP-3 in vivo. ACBP-3 alone or in combination with cisplatin suppressed *xenograft* tumor growth. The antitumor efficacy of cisplatin, when combined with ACBP-3, was enhanced even using half of the normal cisplatin dosage. The study indicated that ACBP-3 inhibited gastric cancer cell growth by suppressing the proliferation of CSCs (Yu et al. 2014).

Guha et al. (2013) developed a special form of TFD (Thomsen-Friedenreich disaccharide), called TFD100, purified from *Pacific cod* that binds to galectin-3, a protein that is over-expressed in prostate cancer cells, and blocks its interaction with the TFD antigen found on the surface of the cells. The TFD100 prevents cancer cells from attaching to the vessel walls, suppresses T-cell death and boosts the immune response (Guha et al. 2013).

Immunomodulatory effect

The association between nutrition and immunity has long been recognized. It has been demonstrated that bioactive peptides derived from various protein sources exert immunomodulatory effects in in vitro and in vivo studies. However, most studies focused on evaluation of the effect of peptides and protein hydrolysates on specific immune systems and only a limited number of investigations examined their impact on nonspecific (innate) immune systems (Shahidi and Zhong 2008).

Bioactive peptides from caseins and whey proteins are also known to have immunomodulatory effect. These peptides can modulate the proliferation of human lymphocytes, downregulate 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 (Korhonen and Pihlanto 2006).

Antimicrobial activity

Bioactive peptides with antimicrobial properties have been identified in a broad variety of natural sources from microorganisms to animals and plants. These peptides display inhibitory effects against food spoilage microbes and a wide range of pathogens in vivo, including bacteria, fungi, virus and eukaryotic parasites. Anti-microbial peptides and foods containing such peptides may be used as antibacterial, antiviral and antifungal agents. Their efficacy is determined by identifying minimum concentrations that inhibit the proliferation of a given group of microorganisms (Najafian and Babji 2012; Dziuba and Dziuba 2014). The effectiveness of these biologically active peptides and the mode of action vary depending on their structural characteristics and show varied selectivity and sensitivity on target microorganisms. In general, animal-derived antimicrobial peptides exhibit inhibitory activity against a much larger spectrum of microorganisms than those produced by bacteria (Rydlo et al. 2006), while the latter show higher efficiency at extremely low concentrations of even nanomolar level (Nagao et al. 2006). However, antimicrobial peptides possess certain common features. Most antimicrobial peptides are composed of less than 50 amino acids with approximately 50 % being hydrophobic amino acids, and often fold into amphipathic 3D structures (Rydlo et al. 2006).

The best investigated antimicrobial peptide is the fragment 17–41 of lactoferrin, more commonly known as lactoferricin. A protection against pathogens has been attributed to α-lactalbumin that involves the release of peptides. Different antimicrobial functions have been attributed to the CMP that is formed during cheese manufacture or digestion from κ-casein. Four peptides GFHI, DFHING, FHG and GLSDGEWQ from bovine meat source were assayed for antimicrobial activity against six pathogenic bacteria, three Gram-positive (*Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*) and three Gram-negative (*Salmonella typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa*) bacteria by Jang et al. (2008). The peptide GLSDGEWQ inhibited the growth of *S. typhimurium*, *B. cereus*, *E. coli* and *L. monocytogenes*. This was the only peptide that inhibited the growth of both Gram-positive and Gram-negative pathogens. GFHI and FHG inhibited the growth of the pathogen *P. aeruginosa*. Yu et al. (2010) have given a comprehensive review that focuses on antimicrobial peptides, including defensins and cathelicidins, found in the blood of animals relevant to the Australasian meat (cattle, sheep, pigs, goats and deer) and poultry (chicken, turkey and ostrich) industries.

Opioids

The first report detailing how a bioactive peptide released from food proteins was regarding the bovine β-casomorphin-7 (YPFPGPI), an opioid peptide from a casein hydrolysate, in late 1970s. 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 number of physiological and pathophysiological 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 behaviours (Guesdon et al. 2006; Shahidi and Zhong 2008).

Many bioactive peptides with opioid activity have been identified and characterized from food proteins and among them milk protein-derived opioid peptides have been most intensively studied. The milk protein-derived opioid peptides have been effective in prolonging gastrointestinal transit time, inhibiting diarrhoea, modulating intestinal transport of amino acids, and stimulating insulin and somatostatin secretion as well as in producing analgesia and modulating social behaviour (Meisel 1998).

There is no documented proof on the generation of opioid peptides from the muscle proteins however, possible opioid sequences, such as Tyr-X-Phe or Tyr-X1-*X*2-Phe are found in the sequences of muscle proteins. Therefore, it should be possible to find opioid peptides in meat proteins by proteolytic treatment. Studies on hemoglobin peptic hydrolyzate have revealed the presence of biologically active peptides with affinity for opioid receptors (Nyberg et al. 1997; Zhao et al. 1997). These peptides were named as hemorphins and were first time isolated from enzymatically treated bovine blood and later were found in brain, plasma, and cerebrospinal fluid.

Anti-genotoxic activity

Park and Hyun (2002) studied the antigenotoxicity potential (the ability to prevent damage to DNA) of hydrolysates from bovine plasma, globulin and albumin by measuring the reduction of DNA damage using the Comet assay. Four different enzymes were used in the study (alcalase, neutrase, pepsin and trypsin) and among them; pepsin proved to be the most effective protease for producing active peptides and the peptic hydrolysate from bovine blood albumin was able to demonstrate the best antigenotoxic effect. Increase in the treated concentrations increased the antigenotoxic activities of the peptic hydrolysate of whole plasma and albumin. The mechanism of action responsible for the antigenotoxicity activity of the peptides was a biological effect resulting from interaction with cells and changing the physiology or metabolism of detoxification rather than a direct chemical inactivation of the carcinogen MNNG.

Anti-obesity

It has been demonstrated that opioid peptides play an important role in the control of food intake, which is implicated to its potential antiobesity activity (bioactive appetite suppressants). Studies have shown that opioid antagonists reduce feeding in most species including humans. Naltrexone has been reported to reduce food intake and eating rate and abolish the stimulation of appetite through palatability in human male subjects. Additionally, antiobesity peptides in β-conglycinin derived from soybean protein (VRIRLLQRFNKRS) and in the CMP as well as hypotriglyceridemic peptides from blood (globin) (VVP; VYP; VTL) are believed to exist.

Lowering of LDL cholesterol and the heightened release of cholecystokinin, an appetite-suppressing hormone has been linked to the total whey protein in the diet (Zhang and Beynen 1993). Combinations of active whey protein fractions or amino acid sequences may be responsible for this bioactivity of total whey protein. This physiological role of total whey protein may be utilized in the development of novel functional foods and suggests a great potential for processed whey products in developing new and lucrative health food markets as functional food ingredients (Regester et al. 1997).

Mineral-binding

Casein-derived phosphopeptides reported as caseinophosphopeptides (CPP) show mineral-binding properties and are involved in the remineralization of tooth enamel as well as in the increased absorption and bioavailability of calcium and other minerals such as zinc, copper, manganese and iron in the intestine. Most CPPs contain a common motif, such as a sequence of three phosphoseryl followed by two glutamic acid residues (Gobbetti et al. 2007). These sequences provide the peptides with the unique capacity to keep Ca, P and other mineral in a solution at intestinal pH. Many phosphopeptides containing the cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu(E)-Glu(E)- have been identified from whole bovine casein (Sharma et al. 2011). The negatively charged side chains, particularly the phosphate groups, of these amino acids represent the binding sites for minerals (Gobbetti et al. 2007). Dephosphorylated peptides do not bind minerals (Berrocal et al. 1989) whereas chemical phosphorylation of α_{s1}- and β-CN increased the binding capacity and the stability of these proteins in the presence of Ca^{2+} (Yoshikawa et al. 1981). These peptides are resistant to further proteolytic attack, by virtue of their highly anionic character, that allows them to form soluble complexes with calcium and prevents the formation of insoluble calcium phosphate (Sato et al. 1986; Berrocal et al. 1989).

Two mineral binding peptides, one with calcium binding property with the sequence VSGVEDVN while other with iron-binding property with the sequence of DLGEQYFKG, were obtained from porcine plasma after hydrolysis with Flavourzyme (Lee and Song 2009a, b). The level of binding ability of the two peptides was relatively similar.

Jung et al. (2005) reported that fish peptides are also capable of accelerating calcium absorption. A fish bone phosphopeptide (FBP) containing 23.6 % of phosphorus was isolated which could bind calcium without the formation of insoluble calcium phosphate. It was suggested that the produce could be used as a nutraceutical with a potential calcium-binding ability (Won-Kyo et al. 2005; Khora 2013).

Antithrombotic activity

Milk and blood coagulation exhibit functional similarities as well as sequence homologies in the fibrinogen g-chain and κ-casein (Jollés and Caen 1991). At the time of milk coagulation by rennin, a peptide split from κ-casein, caseinomacropeptide (CMP), is reported to have peptide sequences, which inhibit the aggregation of blood platelets and the binding of the human fibrinogen γ-chain to platelet surface fibrinogen receptors (Fiat et al. 1993). Chabance et al. (1998) reported two antithrombotic peptides derived from human and bovine κ-caseinoglycopeptides, which were identified in the plasma of 5-day old newborns after breast-feeding and ingestions of cow milk-based formula. C-terminal dodecapeptide of human fibrinogen γ-chain (residues 400–411) and the undecapeptide (residues 106–116) from bovine κ-CN have been reported to be structurally and functionally quite similar (Clare and Swaisgood 2000). Qian et al. (1995) reported that sheep CN-derived κ-caseinoglycopeptide (106–171) decreased thrombin- and collagen-induced platelet aggregation in a dose-dependent manner. Jollès et al. (1986) reported that casoplatelin, the peptide derived from κ-casein, affect platelet function and inhibit both the aggregation of ADP-activated platelets and the binding of human fibrinogen γ-chain to its receptor region on the platelet's surface.

Bacterial growth stimulating properties

A peptide from a bovine hemoglobin hydrolysate with a bacterial-growth-stimulating activity was isolated by reversed-phase high-performance liquid chromatography. The molecular mass and primary structure of the bioactive peptide, determined by fast-atom bombardment mass spectrometry and amino acid analysis, was identical to that of fragment 48–52 (STADA) of the β chain of bovine hemoglobin. Peptide showed growth-stimulating activity on Gram-negative bacteria during microbiological tests in solid media. In the test group comprising enteric bacterial strains, that colonise an environment where hemoglobin is readily available, seven out of ten strains of bacteria were stimulated by the peptide (Zhao et al. 1996).

Organoleptic properties

Peptides also contribute to the organoleptic properties of foods (de Llano Gonzalez and Sanchez 2003; Pihlanto and Korhonen 2003). Foods that involve protein hydrolysis processes during their preparation such as fermentation and ageing show the generation of flavor peptides. Savory flavour peptides are known to be generated from food proteins. An octapeptide with delicious taste was isolated from beef treated with papain (Yamazaki and Maekawa 1980) which was later called as 'beef meaty peptide' or 'savory taste enhancing peptide' (Hau et al. 1997). Umami-taste enhancing peptides were found in chicken protein hydrolysate (Maehashi et al. 1999). Okumura et al. (2004) reported sourness-suppressing peptides generated in cooked pork loins.

Natural products could be made by the addition of protein hydrolysates to enhance the flavor of meat products that plays an important role in replacing synthetic flavor enhancers. Formation of bitter tastes has been identified as a problem associated with food hydrolysates. However, hydrolysates of meat, fish and gelatin are less bitter than those from other food sources (Johanna 2007). Thus, meat proteins have a high potential to produce bioactive peptides and be used as functional ingredients for meat products. Incorporation of these bioactive peptides in meat products in order to enhance the functional value of meat products may not be practical at this point, but meat products with bioactive peptides could open door for a new market since demands for functional foods, especially natural functional foods, is increasing rapidly (Arihara 2006).

Conclusions

For development of various health-promoting functional and designer foods, bioactive peptides of animal origin have attracted increasing interest as prominent candidates. Numerous products based on the health-promoting properties of the bioactive peptides have already struck the market and many products are under development, exploiting the potential of food-derived bioactive peptides. This trend is likely to continue alongside with increasing knowledge about the functionalities of the peptides. Research continues to uncover novel bioactive peptides and to reveal their possible functions and health benefits. Suitable technologies have to be developed to isolate the active peptide fractions from the hydrolysates of various proteins of animal origin and to incorporate them into the model foods that will retain their physiological activity for a required period of time. The systematic synthesis of peptides and peptidomimetics has an important role in finding new bioactive structures and for elucidating structural information on the active conformations (Akai and Alizadeh-Pasdar 2006). Advanced technological tools, like proteomics, are required to assess the mechanisms by which bioactive peptides exert their activities. Furthermore, in silico analysis for structure-activity studies using chemometric methods, like artificial neural networks, are effective and useful for identifying bioactive sequences (Meisel et al. 2006). Creation of structure and sequence databases with the application of computational chemistry will enable bioactive fragments to be searched in the protein chain (Dziuba and Iwaniak 2006). Screening methods need to be developed for the measurement of long term effects in order to ascertain effects of food components that are claimed to promote good health. Relevant indicators

or biomarkers that can predict potential benefits relating to a target function in the body have to be identified (Diplock et al. 2000).

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