OXFORD

Food Quality and Safety, 2017, 1, 29–46
doi:10.1093/fqs/fyx006
Review

Review

Bioactive peptides: A review

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Received 11 November 2016; Revised 28 December 2016; Editorial Decision 30 December 2016.

Abstract

Bioactive peptides (BP) are organic substances formed by amino acids joined by covalent bonds known as amide or peptide bonds. Although some BP exist free in its natural source, the vast majority of known BP are encrypted in the structure of the parent proteins and are released mainly by enzymatic processes. Some BP have been prepared by chemical synthesis. BP play a significant role in human health by affecting the digestive, endocrine, cardiovascular, immune, and nervous systems. BP are considered the new generation of biologically active regulators; they can prevent oxidation and microbial degradation in foods and also improve the treatment of various diseases and disorders, thus increasing the quality of life. The growing interest in BP has incentivized the scientific community and the food industry to exploring the development of new food additives and functional products based on these peptides. The present review highlights the recent findings on the identification, bioassays, and use of BP, as well as their potential use as food additives and in the development of functional products.

Key words: peptides, bioactivity, marine origin, nutraceutical, hydrolysis, fermentation.

Introduction

Bioactive Peptides (BP) (Shahidi and Zhong, 2008; Sharma *et al.*, 2011; Walther and Sieber, 2011) have been defined as specific protein fragments that have a positive impact on body functions or conditions and may influence health (Kitts and Weiler, 2003). Currently, more than 1500 different BP have been reported in a database named 'Biopep' (Singh *et al.*, 2014). BP are organic substances formed by amino acids joined by covalent bonds also known as amide or peptide bonds, whereas proteins are polypeptides with a greater molecular weight (MW). BP and proteins play important roles in the metabolic functions of living organisms and, consequently, in human health. They display hormone or drug-like activities and can be classified based on their mode of action as antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding, and antioxidative.

The amino acid composition and sequence determines the activity of the peptides once that they are released from the precursor protein where they are encrypted. Natural processes within the body are modulated by the interaction of specific amino acid sequences that form part of proteins (Fields *et al.*, 2009). Proteins can be classified as endogenous if they are obtained from amino acids by synthesis within an organism, or exogenous if obtained through the diet or from an external source to an organism, and they represent one of the primary components of the food. Proteins from plant and animal origins are potential sources of a wide range of BP encrypted in their structure (Carrasco-Castilla *et al.*, 2012; Bhat *et al.*, 2015a).

Although the correlation between structure and functional properties is not well established, many BP share some structural features that include a peptide residue length between 2–20 amino acids (Moller *et al.*, 2008), and the presence of hydrophobic amino acids in addition to proline, lysine or arginine groups. BP have also shown to be resistant to the action of digestion peptidases (Kitts and Weiler, 2003).

BP have been considered the new generation of biologically active regulators that can prevent, for example, oxidation and microbial degradation in foods. They can be used for the treatment of various medical conditions, thus increasing the quality of life (Lemes *et al.*, 2016). Recently, functional foods (Haque *et al.*, 2008) and nutraceuticals (Moldes *et al.*, 2017) have received much attention, particularly for the impact that they can have on human health and their use in the prevention of certain diseases. Consequently, considerable

interest has been devoted to the production and properties of BP the past few years (Przybylski *et al.*, 2016).

Even though BP have been identified and isolated from several natural sources, and their activities investigated in many disciplines, the present review is mainly concerned with BP in the context of different food matrices.

Sources

Among the macronutrients present in foods, peptides and proteins are of paramount importance, because they supply the required raw materials for protein biosynthesis and represent a source of energy (Walther and Sieber, 2011; Dziuba and Dziuba, 2014). Also, they are part of an intricate series of organic transformations that occur during the processing and storage of foods that ultimately contribute to their sensory characteristics. In addition to their nutritional value, food proteins, and peptides exhibit distinct biological activities (Hartmann and Meisel, 2007; Moller *et al.*, 2008).

BP are predominantly encrypted inside bioactive proteins (Meisel and Bockelmann, 1999). By far, bovine milk (Torres-Llanez *et al.*, 2005; Korhonen, 2009; Léonil, 2014; Mohanty *et al.*, 2015; Mohanty *et al.*, 2016), cheese (Pritchard *et al.*, 2010), and dairy products (Choi *et al.*, 2012) are the greatest sources of bioactive proteins and peptides derived from foods. However, they can also be obtained from other animal sources such as bovine blood (Przybylski *et al.*, 2016), gelatin (Lassoued *et al.*, 2015), meat, eggs, various fish species such as tuna, sardine, herring and salmon. Some vegetal sources of BP and proteins are wheat (Kumagai, 2010), maize, soy (Singh *et al.*, 2014), rice (Selamassakul *et al.*, 2016), mushrooms, pumpkin, sorghum (Moller *et al.*, 2008), and amaranth (Silva-Sanchez *et al.*, 2008). *In vivo*, encrypted peptides can be liberated during gastrointestinal (GI) digestion by enzymes such as trypsin or by microbial enzymes. *In vitro*, BP can also be released during food processing or ripening by microbial enzymes (e.g. *Lactobacillus helveticus*) (Gobbetti *et al.*, 2002; Meisel, 2005; Korhonen and Pihlanto, 2006; Korhonen, 2007; Dziuba and Dziuba, 2014). BP have been identified and isolated from animal and vegetal sources and are abundantly present in protein hydrolysates and fermented dairy products.

Currently, BP and nutraceutical proteins are being developed to improve human health by preventing or alleviating medical conditions such as coronary heart disease, stroke, hypertension, cancer, obesity, diabetes, and osteoporosis (Gilani *et al.*, 2008; Boelsma and Kloek, 2009).

Peptides from animal sources

Peptides derived from animal proteins have been attributed to different health effects (Bhat *et al.* 2015b). Blood is a valuable source of proteins (circa 20%) and represents a promising source of BP. Although blood disposal is a severe problem for meat processors, serum albumin, the main blood protein has received little attention. In a recent study, serum albumin was hydrolyzed using different concentrations of trypsin and the peptide sequences in the hydrolysates presented the following activities: angiotensin-converting enzyme (ACE) inhibition (antihypertensive activity), DPP-IV inhibition (glucose regulation), and antioxidation (Arrutia *et al.*, 2016a).

Blood obtained from the slaughterhouse is part of the meat production food chain that has not been fully exploited. In a recent study, bovine haemoglobin from the slaughterhouse, blood was subjected to *in vitro* GI digestions, 75 unique peptides were unambiguously identified using low-resolution (LR) liquid chromatography (LC)-MS/MS analysis. The use of high-resolution (HR) liquid chromatography (LC)-MS/MS allowed identifying more than 950 unique peptides (Caron *et al.*, 2016). Haemoglobin fragments can have a profound physiological function. The α- and β-globin chains of haemoglobin provide relatively long peptides containing ca. 30 amino acid residues upon proteolysis. Degradation step coupled with excretion afforded shorter peptides from red blood cells. Both the primary and the secondary proteolysis products were subjected to further stepwise *C*- and *N*-terminal chain shortening, giving rise to families of closely related peptides that are found in animal tissue extracts (Ivanov *et al.*, 1997).

Peptides from vegetal sources

Two excellent works on BP from vegetal sources have been recently published (Malaguti *et al.*, 2014; Rizzello *et al.*, 2016). BP produced during *in vitro* GI digestion of soybean seeds and soy milk have been investigated (Singh *et al.*, 2014). The analysis was performed on extracted protein samples from soybean seeds and milk or directly on untreated soy milk. The results indicated that soybean proteins experimented degradation during GI digestion generating a large number of BP, some with established activity, and some with predicted antimicrobial activity. Endogenous proteases were also used to investigate the presence of peptides (Singh *et al.*, 2014). Peptides found in soy milk samples could be formed during food processing (Capriotti *et al.*, 2015).

Soy hydrolysate and the soy-fermented foods, natto and tempeh, were digested with a variety of endoproteases such as pronase, trypsin, Glu C protease, plasma proteases, and kidney membrane proteases to generate oligopeptides, most likely derived from glycinin, a soy protein. Digestion of natto with pronase provided a peptide with ACE inhibitory activity and a peptide with surface active properties. Likewise, hydrolysis of natto with kidney membrane produced a peptide with ACE inhibitory activity, and a peptide with anti-thrombotic activity which resembles hirutonin, a previously described synthetic thrombin inhibitor (Gibbs *et al.*, 2004).

Cereal grains that have been used in human diets for a long time such as wheat, barley, rice, rye, oat, millet, sorghum, and corn, are a rich source of BP (Malaguti *et al.*, 2014). Scientific evidence has shown the health benefits of consuming whole grains for preventing diseases such as diabetes, cancer, and cardiovascular diseases. Wheat and oat showed the presence of ACE inhibitory peptides and dipeptidyl peptidase inhibitor, as well as peptides with anti-thrombotic, antioxidant, hypotensive, and opioid activities. On the other hand, wheat and rice have proteins with peptidic sequences showing anticancer activity. Wheat and barley showed the greatest diversity and abundance of peptides with potential biological activity among the cereal proteins (Malaguti *et al.*, 2014). Further research is required to establish the mechanism to release the active peptides sequences from cereal grains.

2,5-Diketopiperazines (DKPs) also known as cyclic dipeptides have received considerable attention as bioactive compounds. They can be formed from the *N*-terminal amino acid residues of a linear peptide or protein and have been identified in various foods, particularly in roasted coffee, cocoa, roasted malt, chicken essence, and fermented foods such as beer, distillation residue of awamori, and aged sake. DKPs can also be found in whey protein hydrolysates, and some beverages—therefore DKPs have been considered as a functional component. It has been reported that some DKPs found in the distillation residue of awamori show antioxidant activity (Kumar *et al.*, 2012). Cyclo(-Phe-Phe) present in chicken essence acted as a dual inhibitor of the serotonin transporter and acetylcholinesterase. On the other hand, cyclo(-His-Pro) displayed effects such as

food intake inhibition and body weight reduction in rats, therefore it might influence human biological regulation (Yamamoto *et al.*, 2016).

Food industry produces a large amount of waste. For instance, as a result of olive oil extraction two different kinds of waste materials are generated: the solid waste, that is a combination of olive pulp and stone and an aqueous liquor, constituted by vegetation water, soft olive tissues, and water added during refinement. These residues are particularly polluting products that are not easily biodegradable and difficult to treat. In a recent study, a new strategy for the recovery of waste proteins from olive seed with the potential to produce antioxidant and antihypertensive peptides has been investigated. Enzymatic hydrolysates of olive were prepared by treatment with five different proteases namely alcalase, thermolysin, neutrase, flavourzyme, and PTN. Among them, alcalase was the enzyme that yielded the hydrolysate with the highest antioxidant activity. Fractionated hydrolysates showed a high concentration of short chain peptides, with significantly higher antioxidant and antihypertensive capacities than fractions with higher MWs. All hydrolysates showed antihypertensive capacity, obtaining half maximal inhibitory concentration IC50 values from 29 to 350 μg/ml. Thermolysin was the enzyme that afforded the hydrolysate with the highest ACE inhibitory capacity (Esteve *et al.*, 2015).

The processing of fruits and vegetables generates a significant amount of waste material. These residues can be used for animal feeding; however, the vast majority is discarded. Stones of fruits such as plum (*Prunus domestica L.*) are rich in proteins and may be a cheap source of BP that could be useful for the food and pharmaceutical industries. A method for the extraction of proteins from a residual material from plum involving the use of high intensity focused ultrasound has been developed (González-García *et al.*, 2014). The resultant extracted proteins were digested using alcalase, thermolysin, flavourzyme, and protease P enzymes and antioxidant and ACE inhibitory capacities of the hydrolysates were assayed. Alcalase was the enzyme showing the most promising extract for the isolation of antioxidant and potential antihypertensive peptides. Additionally analysis of the Alcalase hydrolysate by RP-HPLC-ESI-Q-TOF enabled the identification of 13 peptides namely: MLPSLPK, HLPLL, NLPLL, HNLPLL, KGVL, HLPLLR, HGVLQ, GLYSPH, LVRVQ, YLSF, DQVPR, LPLLR, and VKPVAPF, which showed antioxidant and antihypertensive activities.

Peptides from food sources

Milk

Bovine milk, cheese, and dairy products are the greatest sources of bioactive proteins and peptides derived from food (Korhonen, 2009; El-Salam and El-Shibiny, 2013; Lemes *et al.*, 2016; Mohanty *et al.*, 2016). Presumably, this may be one of the primary reasons why milk is required beyond nutrition in the first months of life (Moller *et al.*, 2008).

Milk proteins have a range of biological activities. For instance, immunoglobulins have an immunoprotective effect and lactoferrin (Lf) displays antibacterial activity. Low concentrations of growth factors and hormones, mainly present in colostrum, appear to play a significant role in post-natal development (Park and Nam, 2015).

The major role of milk proteins is to supply amino acids and nitrogen to the young mammals and constitute an important part of dietary proteins for the adult (Sharma *et al.*, 2011). Milk proteins are a rich source of biologically active peptides that are released during GI digestion or food processing (Fitzgerald and Meisel, 2003; Meisel and Fitzgerald, 2003). As an example, opioid peptides that exist in dairy products have pharmacological properties similar to morphine and play an active role in the central nervous system (CNS) (Haque *et al.*, 2008).

Using liquid chromatography-mass spectrometry (HPLC-MS) and tandem mass spectrometry (MS), a large number of medium and low-MW BP (opioid, phosphopeptides) were identified in human milk from mothers of pre- and full-term infants. The formation of many peptides confirms the greater susceptibility of human milk to casein proteolysis compared to bovine milk. Characterization of the peptide sequence, allowed to establish the pathway of casein hydrolysis which leads to the formation of small peptides. It was found that the action of a plasmin-like enzyme acting on specific lysine residues is the primary step in casein degradation. This step is followed by endopeptidases and/or exopeptidases mediated cleavage of the oligopeptides to produce a multiplicity of short peptides differing by one or more amino acid residues. This work reinforces the importance of maternal milk and demonstrates the difficulty to reproduce it artificially. As a consequence of the dynamic nature of maternal milk, a succession of potentially BP is produced in the intestine, which is hard to reproduce in artificial products. Further studies are therefore required to ascertain the role of peptides derived from casein as nutritional and pharmacological factors (Ferranti *et al.*, 2004).

Apart from its importance as a growth factor, Lf, an iron-binding glycoprotein present in the milk of all mammals, has antimicrobial properties and shows immunomodulating effects. Lf and Lf-derived peptides have been reported to influence cytokine production which is involved in immune and inflammatory processes of the body (Moller *et al.*, 2008).

Fermentation of milk proteins using lactic acid bacteria (LAB) is an attractive approach to generate functional foods enriched with BP given the low cost and positive nutritional image associated with fermented milk products (Hayes *et al.*, 2007).

Consumption of fermented milk containing BP has a blood pressure—lowering effect in hypertensive subjects. *L. helveticus* LBK-16H fermented milk containing the biologically active peptides Val-Pro-Pro and Ile-Pro-Pro showed to lower blood pressure in hypertensive rats. Two other peptides (Tyr-Pro and Lys-Val-Leu-Pro-Val-Pro-Gln) purified and characterized from fermented milk also showed ACE inhibitory activity in hypertensive rats (Jäkälä and Vapaatalo, 2010). Antihypertensive peptides have also been found in the whey fraction of milk protein. The tetrapeptides α-lactorphin (Tyr-Gly-Leu-Phe) and β-lactorphin (Tyr-Leu-Leu-Phe) obtained by enzymatic proteolysis from whey proteins α-lactalbumin and β-lactoglobulin, respectively, also reduced blood pressure in hypertensive rats (Sipola *et al.*, 2002).

Peptides isolated and characterized from cow milk proteins show mineral-binding, opioid, ACE inhibitory, immunomodulatory, cytotoxicity, anti-carcinogenic, antibacterial, and anti-thrombotic activities. Although, some variations on the nature of BP depending on the milk protein source, the isolation, and characterization of peptides of different bioactivities from milk protein hydrolyzates and products of buffalo, camel, goat, mare, sheep, and yak milk has been reported (El-Salam and El-Shibiny, 2013).

Daily use of fermented milk containing BP has a blood pressure—lowering effect in hypertensive subjects. A fermented milk product—*L. helveticus* LBK-16H—containing the biologically active peptides valyl-prolyl-proline (Val-Pro-Pro) and isoleucyl-prolyl-proline (Ile-Pro-Pro) showed to lower blood pressure in spontaneously hypertensive rats (SHR). Two other peptides (Tyr-Pro and Lys-Val-Leu-Pro-Val-Pro-Gln) purified and characterized from fermented milk also showed ACE inhibitory activity in SHR. Lactorphin

(Tyr-Gly-Leu-Phe) also reduced blood pressure in normotensive and SHR (Sipola *et al.*, 2002; Jäkälä and Vapaatalo, 2010).

Among the microorganisms that have been used, *Lactobacillus* species, *L. helveticus* strains have a relatively high proteolytic activity. When LBK-16H strain was used, peptides Val-Pro-Pro and Ile-Pro-Pro, which have been shown to possess ACE activity were obtained. Presumably, these peptides are stable and are absorbed because of their small size and carboxyl-terminal proline–proline sequence, which is resistant to peptidase (Seppo *et al.*, 2003). Table 1 shows the amino acid sequence of some BP isolated from milk, as well as their bioactivity.

When a whey protein concentrate was treated with trypsin and the obtained peptides were separated using membrane ultrafiltration/nanofiltration, β-lactoglobulin peptides predominated in the hydrolysate (Arrutia *et al.*, 2016b). In a different work, 69 BP released from casein were identified. Particular attention was paid to β-casomorphins and caseino macro-peptide-derived peptides. These sequences of amino acids were classified mainly as ACE inhibitors, opioid, and antimicrobial peptides (Boutrou *et al.*, 2015).

Donkey milk is a valuable product for the food industry due to its nutraceutical, nutritional, and functional properties. Endogenous peptides derived from donkey milk have been investigated for their antioxidant and ACE inhibitory activities. In a recent study, five peptides were prepared and their retention times and fragmentation patterns compared to the natural occurring homologs. Pure peptide standards were tested *in vitro* tested for the specific bioactivity and as a result of the investigation, the novel endogenous antioxidant peptides, namely EWFTFLKEAGQGAKDMWR and GQGAKDMWR, and two ACE inhibitory peptides, namely REWFTFLK and MPFLKSPIVPF, were successfully validated (Zenezini Chiozzi *et al.*, 2016).

Peptides from the permeate of bovine colostrum after dialysis or those generated by a simulated GI digest have been characterized and tested for bioactivity using murine intestinal (mICc12) cells and their bioactivities compared with the bioactivity of intact colostrum. Remarkably, the most potent BP originated from non-digested colostrum, which had only been subject to endogenous protease activity (Jorgensen *et al.*, 2010).

Egg

Eggs are known as a source of valuable proteins in human nutrition and have been considered an important source of many BP (Wu *et al.*, 2010; Zambrowicz *et al.*, 2011; Bhat *et al.*, 2015a) which may find applications in medicine and food industry (Sun *et al.*, 2016). The identification and characterization of biologically active peptides released *in vitro* or *in vivo* from egg proteins have been achieved, and the results have contributed to change the image of the egg as a new source of biologically active ingredients for the development of functional foods with specific benefits for human health and the treatment and prevention of diseases (Bhat *et al.*, 2015a).

It is now well established that eggs contain numerous substances with potential and demonstrated therapeutic effects, beyond supplying basic nutritional requirements (Zambrowicz *et al.*, 2011). The BP Arg-Val-Pro-Ser-Leu obtained from egg white protein was chemically synthesized and bio assayed to show ACE inhibitory activity, as well as good stability in a simulated GI digestion (Yu *et al.*, 2011).

Research aimed to identify new and existing biological activities of hen egg components will help to define new methods to further improve the value of eggs. Egg white protein powder (EWPP) is a novel egg-derived product that is being increasingly applied in the food processing industry because of its long shelf life. EWPP has been hydrolyzed by three different proteases and the enzymatic hydrolysates sequentially fractionated by ultrafiltration membranes. Among the enzymes that were used alcalase can be considered the best enzyme for the preparation of antioxidant peptides derived from egg white protein (Lin *et al.*, 2011).

The effect of cooking methods and GI digestion on the antioxidant activity of peptides derived from avian egg have been studied. The results suggest that fresh egg yolk has higher antioxidant activity than fresh egg white and whole eggs. Cooking of eggs reduced the antioxidant activity whereas simulated GI digestion increased it. Boiled egg white hydrolysate showed the highest activity and a total of 63 peptides have been identified, which indicates the formation of novel antioxidant peptides. This results suggest the potential role of eggs as a dietary source of antioxidants (Remanan and Yu, 2014).

Three ovomucin hydrolysates have been prepared and desalted and only the desalted alcalase hydrolysate increased the proportion of low-MW peptides which showed anti-inflammatory activity. The showed biological activity was comparable to anti-inflammatory activity in dermal fibroblasts. The anti-inflammatory activity of low-MW peptides was regulated through the inhibition of tumour necrosis-mediated nuclear factor κB pathway. This class of peptides may have potential applications for maintenance of dermal health and treatment of skin diseases (Sun *et al.*, 2016).

Meat

Meat and meat products are traditionally associated with increased risk of cancer, obesity, and other diseases, ignoring the role fact meat plays in human health. BP derived from meat products have the potential for incorporation into functional foods and nutraceuticals. Meat and fish derived peptides have been shown to exhibit antihypertensive effects *in vivo*, along with antioxidant capabilities and other bioactivities such as antimicrobial and anti-proliferative activities *in vitro* (Ryan *et al.*, 2011; Lafarga and Hayes, 2014; Mora *et al.*, 2014; Liu *et al.*, 2016; Ryder *et al.*, 2016). The potential benefits of these compounds to human health has been recently reviewed

Table 1. Some examples of BP from bovine milk proteins (Mohanty *et al.*, 2016).

Source	Peptide (AA sequence)	Bioactivity
Lactobacillus rhamnosus + digestion with pepsin	Asp-Lys-Ile-His-Pro-Phe, Tyr-Gln-Glu-Pro-Val-Leu	ACE inhibitory
Lactobacillus helveticus	Val-Pro-Pro, Ile-Pro-Pro	ACE inhibitory
Lactobacillus GG enzymes + pepsin and trypsin	Tyr-Pro-Phe-Pro, Ala-Val-Pro-Tyr-Pro-Gln-Arg, Thr-Thr-Met-Pro-Leu-Trp	Opioid, ACE inhibitory, immune-stimulatory
Lactobacillus delbrueckii subsp., bulgaricus IFO13953	Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met	Antioxidative
Kluyveromyces marxianus var.	Tyr-Leu-Leu-Phe	ACE inhibitory
β-Casein derived peptides	Lys-Val-Leu-Pro-Val-P(Glu)	ACE inhibitory

(Howell and Kasase, 2010; Stadnik and Kęska, 2015). However, a limited number of food products containing meat derived BP are commercially available (Ryan *et al.*, 2011).

In the first report of an *in vivo* study of antihypertensive activity the novel peptides Arg-Pro-Arg from nebulin and Lys-Ala-Pro-Val-Ala and Pro-Thr-Pro-Val-Pro from titin, identified in the digest of pork meat after oral administration to SHR. The peptide Arg-Pro-Arg showed the greatest activity *in vivo*. These results suggest that pork meat constitute a source of bioactive compounds that could be utilized in functional foods or nutraceuticals (Escudero *et al.*, 2012).

Exogenous peptides

Marine organisms (Ngo *et al.*, 2012) are an important source for BP that have been employed for the treatment of various diseases (Kang *et al.*, 2015; Manikkam *et al.*, 2016). Chemical diversity is the result of the highly dynamic marine environment, and it represents an unlimited resource of new active substances with potential use as bioactive products. Marine organisms such as fish, shellfish mollusks, marine processing waste, and crustaceans are abundant sources of a myriad of structurally diverse bioactive organic compounds (Aneiros and Garateix, 2004; Lee *et al.*, 2012; Rustad and Hayes, 2012; Cheung *et al.*, 2015; Jo *et al.*, 2017). In Table 2, the structures of some short peptides of marine origin, as well as the corresponding activity are represented. A well-documented evidence of their potential for human health (Fan *et al.*, 2014; Ngo *et al.*, 2012) which includes activities as antihypertensive (Kim *et al.*, 2012), antioxidant (Ngo and Kim, 2013), antimicrobial (Kang *et al.*, 2015; Falanga *et al.*, 2016), anticoagulant, anti-diabetic (Manikkam *et al.*, 2016), anticancer, immunostimulatory, calcium-binding, hypocholesteremic and appetite suppression has incentivized the interest of these compounds as functional food ingredients (Harnedy and Fitzgerald, 2012).

BP isolated and identified from crustaceans, regulate a large number of physiological functions, including colour change, heart activity, exoskeletal and visceral muscles, metabolic function, development, metamorphosis, and reproduction. Proteins derived from these marine organisms represent a unique source of proteins that can be used as raw materials for the generation of biofunctional peptides (Kim *et al.*, 2012; Lee *et al.*, 2012; Kim and Kim, 2013; Ngo and Kim, 2013; Kang *et al.*, 2015; Falanga *et al.*, 2016).

The chemistry and biological activities of BP obtained from marine algae, sponges, tunicates, ascidians, coelenterates, and mollusks have been investigated (Cheung *et al.*, 2015). Marine algae have high protein content—up to 47% of the dry weight—depending on the species, which has increased the interest on these organisms as a source of BP. *In silico* proteolysis and quantitative structure–activity relationship studies of marine algae-derived BP, as well as *in vivo* evaluation and novel technologies in BP studies and production have been reviewed (Fan *et al.*, 2014).

Macroalgae are a group of marine organisms that have developed complex and unique metabolic pathways producing a surplus of bioactive organic compounds. These include proteins, linear peptides, cyclic peptides and depsipeptides, peptide derivatives, amino acids, and amino acid-like components. Some BP derived from macroalgae proteins have been characterized; however, macroalgal proteins still represent an unexplored source of BP that can be used as functional foods to provide specific health benefits and disease-preventing properties mainly antihypertensive (Harnedy and Fitzgerald, 2012).

Pharmacological Properties and Health Benefits

Currently, the relationship between chemical structure and activity of a peptide can not be predicted. The activity of a peptide depends on its structure, i.e. the amino acid composition, the type of *N*- and *C*-terminal amino acid, the length of the peptide chain, charge character of the amino acids forming the peptide, the hydrophobic/hydrophilic characteristics of the amino acid chain, among others. For instance, peptides with higher ACE inhibitory activity usually have aromatic or basic *N*-terminal amino acids, higher quantity of hydrophobic and positively charged amino acids in *C*-terminal (Li and Yu, 2015).

To be considered bioactive, a dietary component should impart a measurable biological effect at a physiologically level. This bioactivity must have the potential to affect health in a beneficial way, which excludes potentially damaging effects such as toxicity, allergenicity, and mutagenicity (Moller *et al.*, 2008).

In Table 3, the amino acids sequence of some peptides, along with the corresponding activity are presented.

Antioxidants

Peptides derived from milk proteins have shown antioxidant properties that prevent peroxidation of essential fatty acids. For instance, the addition of a Leu or Pro residue to the *N*-terminus of a His-His, dipeptide will enhance antioxidant activity and facilitate further synergy with non-peptide antioxidants [e.g. butylated hydroxytoluene (BHT)]. On the other hand, it has been demonstrated, that digestion of casein produces phosphorylated peptides that exhibit both hydrophilic and lipophilic antioxidant activity due to both metal ion sequestering and quenching of ROS (Clare and Swaisgood, 2000).

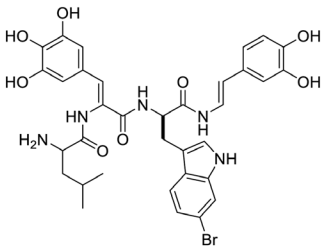
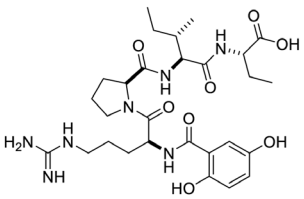
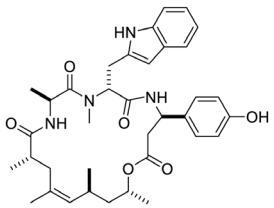
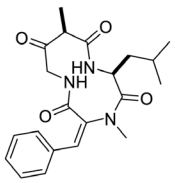
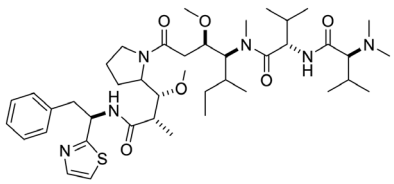
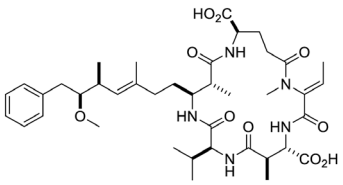
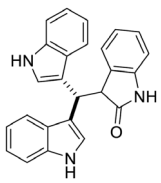
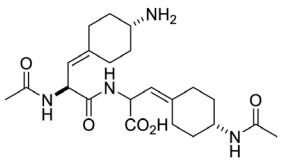
The fish processing industry produces more than 60% by-products as waste. To use these wastes, and to add value to several underutilized fish species, protein hydrolysates from fish proteins are being prepared using enzymes. These fish protein hydrolysates contain small BP with antioxidant activity (Elias, 2008). The antioxidant potential of protein hydrolysates depends on the amino acid composition and on the disruption of the tertiary structure of parent proteins by enzymatic hydrolysis that results in increasing the solvent accessibility of oxidatively labile amino acid residues.

The antioxidant activities of water-soluble fractions of a Spanish dry-cured ham extract of a fractionated peptide extract by size-exclusion chromatography have been described. Some of the fractions exhibited great 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity, varying from 39 to 92% as well as superoxide ion extinguishing ability with values from 41.7 to 50.3% of the antioxidant activity, suggesting the presence of peptides with antioxidant activity. The combination of antihypertensive and antioxidant capacities could help to reduce the adverse effect of NaCl, an ingredient commonly used in these products (Escudero *et al.*, 2012).

Soy peptides have shown increased antioxidant activities (Singh *et al.*, 2014) compared to intact proteins (Peña-Ramos and Xiong, 2002). Soy protein hydrolysates prepared from native and heated soy protein isolates using different enzymes had varying degrees of hydrolysis (1.7–20.6%) and antioxidant activities (28–65%) (Peña-Ramos and Xiong, 2002).

The antioxidant peptides Leu-His-Tyr, Leu-Ala-Arg-Leu, Gly-Gly-Glu, Gly-Ala-His, Gly-Ala-Trp-Ala, Pro-His-Tyr-Leu and Gly-Ala-Leu-Ala-Ala-His were obtained from sardinelle (*Sardinella aurita*) industrial wastes generated using crude enzyme extract from sardine (*Sardina pilchardus*). The first tripeptide displayed

Table 2. Examples of short peptides from marine origin.

Peptide	Peptide
Celenamide B 	Nazunamide A 
Linear peptide with alkaloid-related activity isolated from the sponge *Cliona celata*. Fusetani (1993).	Linear peptide from the sponge. *Theonella swinhoei and shows* anticoagulant activity, thrombin inhibitor. Fusetani and Matsunaga (1993).
Jasplakinolide 	Tentoxine 
Cyclic depsipeptide isolated from the marine sponge *Jaspis johnstoni*, induces apoptosis associated with caspase-3 activation and a decrease in Bcl-2 protein expression. Zhen *et al.* (2011).	Cyclopeptide isolated from the pathogenic fungus *Alternaria tenuisness*, deshidrodepsipeptide with potential herbicidal activity. Jiménez *et al.* (2003).
Dolastin 	Mutoporin 
Peptide isolated from the mollusk *Dolabella auricularia*. Anticancer activity against the P388 cell line; inhibits tubulin polymerization and tubulin dependent GTP hydrolysis. Aneiros (2004).	Cyclopeptide isolated from the Papua New Guinean *Theonella swinhoei*, with potent inhibitory activity against protein phosphatase 1 and cytotoxic activity against P388 cell line. De Silva (1992).
Trisindoline 	Radiosumine 
Peptide from the marine sponge *Hyrtios altum*. Shows antibacterial activity against *Escherichia coli*, *Bacillus subtilis* and *S. aureus*. Kobayashi (1994).	Dipeptide with non-proteinogenic amino acids shows activity as a protease inhibitor. Isolated from the blue-green freshwater algae *Plectonema radiosum*. Noguchi *et al.* (2002).

the highest DPPH radical scavenging activity and, presumably, the presence of the amino acids His-Tyr sequence could contribute significantly to the antioxidant activity of the peptides (Bougatef *et al.*, 2010).

Enzymatic hydrolysates from wheat germ protein (Zhu *et al.*, 2006) and α and β-lactoglobulin (Hernandez-Ledesma *et al.*, 2008) possess antioxidant and free radical-scavenging activities. Antioxidant proteins and peptides have also been identified in egg (Sakanaka and Tachibana, 2006), potato, and gelatin (Je *et al.*, 2005).

The presence of antioxidant peptide segments in proteins may help to explain why dietary protein intake can promote animal and human health beyond the standard nutritional benefits exerted (Elias *et al.*, 2008). Digestion studies *in vitro* have provided evidence that a mixture of peptides (2–4 amino acid residues) obtained from certain

Table 3. Examples of peptides with known activity.

Sequence	Origin	Activity	Reference
FSDKKIAK		Antimicrobial	Capriotti *et al.* (2016)
EQLTK		Antimicrobial	Wada and Lönnerdal (2014)
LKP		Antihypertensive, ACE	Erdmann *et al.* (2008)
AKYSY		Antihypertensive, ACE	Harnedy and FitzGerald (2011)
KRQKYD		Antihypertensive, ACE	Lafarga (2014)
KRPKHPIKH		Antihypertensive, ACE	El-Salam (2013)
SVPQPK		Antihypertensive, ACE	Hernández-Ledesma *et al.* (2007)
VVYPWYQ		Antihypertensive, ACE	Lee *et al.* (2012)
VECYGPNRPQF		Antioxidant	Ricci-Cabello *et al.* (2016)
WYSLAMAASDI		Antioxidant, radical scavenging	
LVNPHDHQNLK		Antioxidant	Capriotti (2015)
WYSLAMAASDI		Antioxidant	Nongonierma and Fitzgerald (2015)
KVREGTTY		Antihypertensive	Bhat *et al.* (2015b)
VPPIPP		Antihypertensive	Korhonen (2009)
IBYW		Hipoglucemiante	Guerin *et al.* (2016)
KLPGF		Hipoglucemiante	Patil (2015)
GGGGYPMYPLR		Inmunpmodulatory	Agyei *et al.* (2016)
GLF		Inmunpmodulatory	Kamau (2010)
GLLVDLL		Anticancer	Sanjukta and Rai (2016)
YGLF		Opioide	Meisel (1999)
LDAVNR		Anti-inflammatory	Fan (2014)
NIGK		Platelet activating inhibition	Fan (2014)

food proteins using human digestive enzymes under physiological conditions possess potent antioxidant activity (Zhu *et al.*, 2008).

Shellfish is another potential source for antioxidant peptide production in the GI system (Ngo and Kim, 2013). The GI digests of oyster (*Crassostrea gigas*) yielded an active 1.6 kDa peptide with the amino acid sequence of Leu-Lys-Gln-Glu-Leu-Glu-Asp-Leu-Leu-Glu-Lys-Gln-Glu. This peptide inhibited peroxidation of lipids and neutralized hydroxyl and superoxide radicals (Ngo and Kim, 2013).

Alaska pollock frame protein is normally discarded as an industrial by-product in the processing of fish. The frame protein hydrolysatpe (APH) was fractionated and the fraction labelled as APH-V exhibited the highest antioxidative activity. The fraction was further purified to afford the peptide Leu-Pro-His-Ser-Gly-Tyr (Je *et al.*, 2005).

In a different study, the peptide Leu-Val-Gly-Asp-Glu-Gln-Ala-Val-Pro-Ala-Val-Cys-Val-Pro (1.59 kDa)—with potent antioxidant activity—was isolated from the GI digest of mussel (*Mytilus coruscus*) muscle protein (Jung *et al.*, 2007). This peptide exhibited higher protective activity against lipid peroxidation than ascorbic acid and α-tocopherol at comparable dosage levels. Another peptide with 16 amino acid residues (1.8 kDa) showing strong inhibition of lipid peroxidation was isolated from the peptic hydrolysate of hoki (*Johnius belengerii*) frame protein (Kim *et al.*, 2007).

Endogenous antioxidant peptides

Oxidation is one of the leading causes of diseases and pathogenesis in humans. Glutathione, a tripeptide (γ-Glu-Cys-Gly), protects cells from free radicals. The dipeptide carnosine (β-alanyl-histidine), and its related dipeptides anserine (β-alanyl-1-methylhistidine) and balenine (β-alanyl-3-methylhistidine), have high antioxidant and pH-buffering properties in muscle cells.

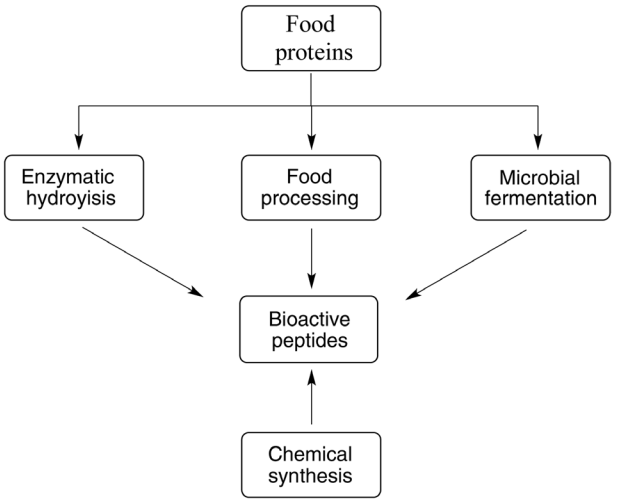


Figure 1. Schematic representation of the production of bioactive peptides (adapted from Danquah and Agyei, 2012).

Melatonin, *N*-acetyl-5-methoxytryptamine, can protect cells against oxidation (Xiong, 2010). The endogenous dipeptide cyclo(His-Pro) that is present in the CNS and several body fluids and the GI tract, has a neuroprotective role. *In vitro* studies on rats have demonstrated that cyclo(His-Pro) enhances the cellular antioxidant capacity as well as the expression of small heat shock proteins. Additionally, there is some evidence that cyclic dipeptide has a role in ameliorating diabetes (Minelli *et al.*, 2008).

Antioxidant activity of peptides in food systems

Peptides derived from milk proteins have also shown antioxidant activity to prevent peroxidation of essential fatty acids. The addition of a Leu or Pro residue to the *N*-terminus of a His-His, dipeptide will enhance antioxidant activity and facilitate further synergy with non-peptide antioxidants (e.g. BHT) (Kitts and Weiler, 2003). It has been demonstrated that many peptides and protein hydrolysates from plant and animal origin possess antioxidant activity. For example, peptides derived from soy protein (Wang and De Mejia, 2005; Singh *et al.*, 2014), whey protein (Pihlanto-Leppälä, 2000; Saito, 2008) casein, (Phelan *et al.*, 2009), corn protein (Kong and Xiong, 2006; Li *et al.*, 2008), potato protein (Wang and Xiong, 2005) (Pihlanto *et al.*, 2007), wheat protein (Kumagai, 2010), gelatin (Mendis *et al.*, 2005), egg protein (Liu *et al.*, 2010; Bhat *et al.*, 2015a) and muscle protein (Ryan *et al.*, 2011; Lafarga and Hayes, 2014) showed antioxidant properties (Liu *et al.*, 2016).

β-Lactoglobulin exhibited antioxidant activity in Brij-stabilized oil-in-water emulsions; however, its chymotryptic hydrolysate was more efficient for the inhibition of lipid peroxidation (Elias *et al.*, 2006).

Antioxidant peptide fragments containing tyrosine and methionine had substantial radical-scavenging activity and played a significant role in the overall antioxidant activity of the protein hydrolysates (Torkova *et al.*, 2015). The antioxidant activity of several protein hydrolysates and peptide mixtures has been tested *in situ* and some of them have been already used as ingredients in commercial food processing.

Whey, casein, soy, potato, and yolk protein hydrolysates have been shown to inhibit lipid oxidation in muscle foods (Wang and Xiong, 2005). A total of 21 peptides were identified from the hydrolysate of bivalve mollusks *Mactra veneriformis* and the sequences established using MS/MS fragmentation data. These peptides were chemically synthesized, and showed antioxidant activity in radical

scavenging assays. The strategy utilized in the investigation proved to be suitable for quick, sensitive, and accurate analysis of complex protein hydrolysates (Liu *et al.*, 2015).

Proteins of goat milk were hydrolyzed by pepsin into caseins (GCP), whey proteins (GWP) and protein fractions containing multiple soluble peptides. Proteins and peptides derived from goat milk have also received increased attention, particularly the BP released from the parent proteins by digestive enzymes that showed reduced allergenicity compared to bovine milk. The generated peptides were examined for radical scavenging activities. The hydrolysates of whey (P-GWP) and casein (P-GCP) proteins exhibited potent superoxide anion ($O_2 \cdot -$) scavenging activity. The results demonstrate that soluble peptides obtained by digestion with pepsin possess remarkable ability to scavenge superoxide radicals and thus providing an interesting opportunity for their potential candidacy as antioxidant BP (Ahmed *et al.*, 2015).

Antimicrobial

Antimicrobial peptides (AMPs) are an abundant and diverse group of molecules that are produced by many tissues and cell types in a variety of invertebrate, plant, and animal species. Their amino acid composition, amphipathicity, cationic charge and size allow them to attach to and insert into membrane bilayers (Brogden, 2005). These peptides are involved in the inhibition of cell growth and in the killing of several microorganisms, such as bacteria and fungi. Antimicrobial peptides are usually below a MW of 10 kDa and encoded within the sequences of native protein precursors, may also be generated *in vitro* by enzymatic hydrolysis (Kim and Wijesekara, 2010). AMPs constitute a promising alternative as therapeutic agents against various pathogenic microbes (Cruz *et al.*, 2014). More than 60 peptide drugs have reached the market for the benefit of patients and approximately 140 peptide therapeutics are currently being evaluated in clinical trials (Fosgerau and Hoffmann, 2015).

AMPs can be classified into three families according to different structural features: 1. α- helical linear peptides, 2. disulfide-bridged cyclic and open-ended cyclic peptides, and 3. peptides whose primary structures have a high content of some amino acid residues (e.g. proline, glycine or histidine rich). Most of these peptides adopt an amphipathic structure with both cationic and hydrophobic properties that facilitate their interaction with anionic cell walls and membranes of microorganisms (Pimenta and De Lima, 2005).

Four linear AMPs were isolated from the venom of the primitive scorpion *Opisthacanthus madagascariensis*. Other antimicrobial α-helical peptides were described from the venom of the scorpions *Hadrurus aztecus* (hadrurin), *Opistophtalmus carinatus* (opisto-porin 1 and 2), *Parabuthus schlechteri* (parabuto-porin), and *Pandinus imperator* (pandinin 1 and 2). From Hymenoptera venom, antimicrobial peptides have been described in wasps (19), bees, and ants. Antimicrobial α-helical peptides have also been described in the venoms of spiders. AMPs have been described from the venom of wolf spiders *Lycosa singoriensis*, *Lycosa carolinensis*, *Lycosa erythrognatha*, and *Oxyopes kitabensis* (Pimenta and De Lima, 2005).

Marine-derived antimicrobial peptides are well described in the haemolymph of the many marine invertebrates (Tincu and Taylor, 2004).

Casein Derived Antibacterial Peptides show inhibitory activity against *Streptococcus mutans*, *Streptococcus sanguis, Porphyromonas gingivalis*, *Streptococcus sobrinus*, *Sthaphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*. For instance, after digestion with chymosin enzyme, casein-α_{s1} provides two peptides known as caseicin A and caseicin B that inhibit several pathogens (*Staphylococcus, Sarcina, Bacillus subtilis, Diplococcus pneumoniae, and Streptococcus pyogenes*). On the other hand, Lf a Whey Protein Derived Antibacterial Peptide shows bacteriostatic effects *in vivo* and *in vitro* against *Bacillus stearothermophilus, B. subtilis, Clostridium spp., Haemophilus influenza, Streptococcus mutans, Vibrio cholerae, E. coli, and Legionella pneumophila* (Mohanty *et al.*, 2015).

A small anionic antibacterial peptide XLAsp-P1 was isolated from the skin of *Xenopus laevis*. XLAsp-P1 has a remarkable *in vitro* potency against Gram-positive and Gram-negative bacteria and potent inhibitory activity against breast cancer cells. The use of transmission electron microscopy, allowed to understand the mechanism of action of this novel peptide. The antimicrobial activity is based on the destruction of the cell membrane (Li *et al.*, 2016).

Bovine coagulated blood, a slaughterhouse by-product, has been described as a rich source of antimicrobial peptides. The study identified a 137–141 fragment of hemoglobin (Thr-Ser-Lys-Tyr-Arg), a small (653 Da) hydrophilic antimicrobial peptide that in addition presented potential as meat preservative reducing the lipid oxidation about 60% to delay meat rancidity. The 137–141 peptide also inhibited the microbial growth under refrigeration during 14 days. These antimicrobial effects were close to those of the BHT (Przybylski *et al.*, 2016).

Immunomodulatory

Immunomodulatory peptides derived from hydrolysates of rice and soybean proteins act to stimulate reactive oxygen species (ROS), which triggers non-specific immune defence systems. The anterior pituitary (AP) contains a variety of BP such as: brain-gut peptides, growth factors, hypothalamic releasing factors, posterior lobe peptides, opioids, and various other peptides. The release of peptides from the AP cell, the synthesis, storage and release have been described. Since AP peptides are produced in low quantities, endocrine activity has not been established. There is some evidence for paracrine, autocrine, or intracrine roles in growth, differentiation, and regeneration, or in the control of hormone release (Houben and Denef, 1994). Ngo *et al.* (2012) reported that marine organisms are an important source for BP that have been employed for the treatment of various diseases (Kang *et al.*, 2015; Manikkam *et al.*, 2016).

Celiac disease is a genetic autoimmune disorder consisting in the damage of the small intestine after the ingestion of gluten. The roles of the many BP in the pathogenesis of celiac disease remain unclear. An increase in insulin concentrations in individuals with celiac disease has been observed, but further studies are required to determine whether increased insulin concentrations is related to an increased risk for hyperinsulinemia (Janas *et al.*, 2016).

Anti-inflammatory

Proteins and peptides from egg, milk, soy, and plant sources have shown anti-inflammatory properties. Ovotransferrin is an egg white protein well known for its antibacterial activity (Wu *et al.*, 2010). It is often present in large amounts in chickens, during inflammation and infection processes. This protein also has immunomodulating effects on chicken macrophages and heterophil-granulocytes and can inhibit proliferation of mouse spleen lymphocytes (Xie *et al.*, 2002). Immunomodulatory peptides derived from hydrolysates of rice and soybean proteins act to stimulate ROS, which triggers non-specific immune defence systems.

The molecular diversity of marine peptides, as well as information about their anti-inflammatory properties and mechanisms of action, have been described, along with the anti-inflammatory effects

of novel BP from sponges, bacterium, and microalgae (Kim *et al.*, 2010).

The anti-inflammatory, anti-hemolytic, antioxidant, anti-mutagenic, and antimicrobial activities of crude extracts and peptide fractions obtained from fermented milks with specific *Lactobacillus plantarum* strains was carried out. Crude extracts showed higher activity than both peptide fractions in most of the activities assessed. In particular, *L. plantarum 55* crude extracts or their fractions showed higher anti-inflammatory that diclofenac sodium equivalents. These results provide valuable evidence of multifunctional role of peptides derived of fermented milk by the action of specific *L. plantarum* strains (Aguilar-Toalá *et al.*, 2017).

The protein SV-IV that is synthesized by rat seminal vesicle (SV) epithelium has shown anti-inflammatory activity. In an attempt to identify the shortest peptide possessing the full anti-inflammatory activity of the native protein, a number of SV-IV oligopeptides, whose sequences were derived from the *N*-terminal segment of the protein, were prepared by Fmoc chemistry and their anti-inflammatory properties were evaluated. The results strongly suggested that the anti-inflammatory activity of SV-IV was mainly related to amino acids 8 ± 16 of the native protein (Ialenti *et al.*, 2001).

Purification and identification of anti-inflammatory peptides derived from simulated GI digests of velvet antler protein (*Cervus elaphus Linnaeus*) has been performed. Sequential chromatography-MS (LC-MS/MS) was used to purify and identify four anti-inflammatory peptides (VH, LAN, AL and IA) from SGD-VAP. Among the peptides, IA showed the strongest anti-inflammatory activities at 200 mg/ml. These results suggested that the peptides derived from velvet antler protein could potentially be used as a promising ingredient in functional foods or nutraceuticals against inflammatory diseases (Zhao *et al.*, 2016).

Antihypertensive

Antihypertensive peptides, also known as ACE inhibitors have been derived from milk, corn, and fish protein sources (Kim *et al.*, 2012). ACE is essential for the regulation of blood pressure (Borer, 2007). ACE catalyzes the transformation of angiotensin I to potent vasoconstrictor angiotensin II and the degradation of the vasodilator, bradykinin. Since angiotensin II raises BP, therefore inhibition of ACE can decrease BP. Structure-activity correlations among different peptide inhibitors of ACE indicate that binding to ACE is strongly influenced by the *C*-terminal tripeptide sequence of the substrate. ACE appears to prefer substrates or competitive inhibitors which mainly have hydrophobic (aromatic or branched side chains) amino acid residues at the three *C*-terminal positions (Gobbetti *et al.*, 2002).

Several tripeptides that inhibit ACE have been isolated from foods. The milk-derived bioactive tripeptides Val-Pro-Pro and Ile-Pro-Pro reduced blood pressure in mildly hypertensive subjects (Tuomilehto *et al.*, 2004). Two tripeptides that inhibit ACE, namely Ile-Pro-Pro and Val-Pro-Pro, were isolated from sour milk fermented with *L. helveticus* and *Saccharomyces cerevisiae* (Moller *et al.*, 2008). There is some evidence that the ingestion of sour milk fermented by *L. helveticus* that contain ACE inhibitory tripeptides lowers BP modestly. Long-term administration of Ile-Pro-Pro and Val-Pro-Pro has been shown to prevent the development of hypertension in rats and to reduce BP by a single oral administration (Tuomilehto *et al.*, 2004).

Since ACE inhibitory peptides can be obtained by the action of enzymes on complete proteins *in vitro* during food processing and *in vivo* through GI digestion, peptides that inhibit ACE may be generated in or incorporated into functional foods to develop useful health products. Some products containing peptides with ACE inhibitory properties are being investigated, or some are already in the market, but further studies to validate their efficiency are required (Murray and FitzGerald, 2007).

In a different study, rice protein was hydrolyzed by alcalase and the resulting hydrolysate was tested for ACE inhibitory activity *in vitro*. The antihypertensive effect of rice protein hydrolysate was also investigated in SHR showing an IC50 value of 0.14 mg/ml. A potent ACE inhibitory peptide with the amino acid sequence of Thr-Gln-Val-Tyr (IC50, 18.2 μM) was isolated and identified from the hydrolysate. Single oral administration of Thr-Gln-Val-Tyr at a dose of 30 mg/kg of body weight also significantly decreased blood pressure in SHR (Li *et al.*, 2007).

The commercially available Food for Specified Health Uses (FOSHU) products containing small peptides: tryptic hydrolysate of casein, Katsuobushi oligopeptide, the aqueous extract from Mycoleptodonoide saitchisonii, sour milk, sardine peptide, seaweed peptides, and sesame peptides inhibit the ACE enzyme. However, the efficacy of peptide intake for borderline hypertensives still requires further investigation.

For a long time, macroalgae have been a key component of the diet of East Asian populations. It is also a source of functional and technological ingredients in the food, pharmaceutical, and cosmetic industries (Pal *et al.*, 2014). The protein from the red alga *Palmaria palmate* was extracted and hydrolyzed with the enzyme papain to generate renin inhibitory peptides, whose sequences were elucidated. The renin inhibitory peptide Ile-Arg-Leu-Ile-Ile-Val-Leu-Met-Pro-Ile-Leu-Met-Ala was isolated from one of the fractions and chemically synthesized. The synthesized peptide was also subjected to *in silico* cleavage analysis using the computer program Expasy peptide cutter and hydrolyzed using enzymes found in the GI tract to release dipeptides and tripeptides. These results suggest that this propeptide and the dipeptides and tripeptides freed from it may cross the lumen into the bloodstream and potentiate an antihypertensive effect (Fitzgerald *et al.*, 2012).

The cereals wheat, oat, barley and rice have been evaluated for BP showing high occurrence frequencies of ACE-inhibitor peptides as well as of dipeptidyl peptidase-inhibitor and anti-thrombotic, antioxidant, hypotensive, and opioid activity. Wheat and rice proteins presented sequences with anticancer activities. Wheat and barley showed the greatest diversity and abundance of potential biological activity among the cereal proteins. Further research needs to be conducted to learn how these biologically active peptide sequences are released from cereal grains (Cavazos and Gonzalez de Mejia, 2013).

A few BP have been tested in human trials. The available data indicates that bioactive tripeptides with antihypertensive activities reduce the risk of elevated blood pressure in subjects with moderate hypertension (Cicero *et al.*, 2013). BP can be used to design prevention strategies. Milk BP may be employed in the prevention of risks in metabolic syndrome (MS) and its complications via the regulation of blood pressure, the uptake of free radicals, and the control of food intake.

Studies on potentially hypotensive milk protein hydrolysates illustrate the significant difference between allergenicity and immunogenicity. Despite the existence of data on the relationship between the structure of food proteins and peptides derived from food, the assessment of the allergenic properties of products derived from an allergenic source is not straightforward (Reddi *et al.*, 2012).

Cytomodulatory

A large number of proteins and peptides of plant and animal origin are known to exhibit cytotoxic effects. There is evidence that many

cytotoxic compounds described in the literature exclusively affect malignant cells leading to the assumption that a cancer protective effect could exist for such bioactive proteins and peptides. All the constituents that are responsible for the allergenicity (Hartmann *et al.*, 2007) of foods are proteinaceous in nature. Some protein breakdown products, i.e. peptide fragments, may conserve part of the allergenicity of the native protein and thus can also be considered as allergens (Ladics and Selgrade, 2009). Despite the existence of data on the relationship between the structure of food proteins and peptides derived from food, the assessment of the allergenic properties of products derived from an allergenic source, is not straightforward (Reddi *et al.*, 2012).

Opioid activity

The endogenous ligands for opioid receptors are peptides, also known as endorphins. These peptides share the common *N*-terminal sequence of Tyr-Gly-Gly-Phe-(Met or Leu), which has been termed the *opioid motif*; this is followed by various *C*-terminal extensions yielding peptides ranging from 5 to 31 residues in length (Aldrich, 2009). Due to the importance of mu opioid receptor (MOR) agonists such as morphine as analgesics, the primary focus has been for the treatment of pain. The emphasis for opioid peptide analog development has been to induce analgesia through activation of opioid receptors in the CNS.

In vivo studies of opioid peptides in humans and animals have shown that analogs of dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), an endogenous opioid peptide that exhibits high selectivity for MOR, such as the dermorphin tetrapeptide analog ADAMB (H_2NC(NH)-Tyr-D-Arg-Phe-MebAla-OH) was designed by incorporating modifications, including *N*-terminal quanidylation, from several dermorphin tetrapeptides that exhibited weak oral analgesic activity. ADAMB was 4- and 38-fold more potent as an analgesic than morphine following oral and s.c. administration (Aldrich and McLaughlin, 2012).

BP derived from milk proteins may function as exorphins or formones (food hormones). Although this role needs further clarification, pharmacological properties similar to morphine and naloxone inhibitable properties have been shown (Gobbetti *et al.*, 2002). The α- and β-casomorphins and lactorphins act as opioid agonists, while casoxins act as opioid antagonists. Casomorphins may produce analgesia, modulate social behaviour, influence postprandial metabolism by stimulating the secretion of insulin and somatostatin, and may influence GI absorption of nutrients by prolonging the GI transit time and exerting an antidiarrhoeal effect (Meisel and Schlimme, 1990).

Peptides with opioid activities (Aldrich, 2009; Yamamoto *et al.*, 2003) can be derived from wheat gluten or casein, following digestion with pepsin. Exorphins or opioid peptides derived from food proteins such as wheat and milk (e.g. exogenous sources) have similar structures to endogenous opioid peptides, with a tyrosine residue located at the amino terminal or bioactive site (Kitts and Weiler, 2003; Yamamoto *et al.*, 2003; Aldrich, 2009).

Mineral-binding

Peptides as calmodulin inhibitors

A potential target for food protein-derived BP is calmodulin (CaM), a protein that plays important roles in maintaining physiological functions of cells and body organs (Chung *et al.*, 2000). CaM is a calcium-binding protein formed by 148 amino acid residues with a weight of 16.7 kDa. This protein plays multifunctional roles in the translation of intracellular messages (Klee and Vanaman, 1982). Some of the functions of CaM include calcium-dependent cell division, cell proliferation, and neurotransmission (Rasmussen and Means, 1987). Excessive levels of CaM can have a detrimental effect on the body and may lead to the development of chronic diseases such as cancer.

The calmodulin-binding peptides LKKISQRYQKFALPQY; VYQHQKAMKPWIQPKTKVIPYVRY; VYQHQKAMKPWIQPKTKVIPYVRYL; AMKPWIQPKTKVIPYVRYL; KPWIQPKTKVIPYVRYL have been isolated from peptic hydrolysate of bovine casein and showed to inhibit CaM-dependent phosphodiesterase (CaM-PDE).

Flaxseed proteins have hydrophobic and positively charged amino acids that could enhance the production of CaM-binding peptides through enzymatic hydrolysis. Hydrolysis of flaxseed proteins with alcalase, afforded low-MW peptides (Omoni and Aluko, 2006). Similarly, hydrolysis of pea proteins with alcalase produced CaM-binding peptides (Li and Aluko, 2010).

Metabolic syndrome

MS consists of a deadly quintet of factors, namely diabetes, centripetal obesity, hypertension, dyslipidemia (elevated triglycerides, dense low-density lipoproteins, and low high-density lipoproteins), and alterations in the thrombotic potential that are related to hyperinsulinemia and insulin resistance (Hollander and Mechanick, 2008).

Peptides structurally related to the insulin receptor (IR)-binding protein mcIRBP-19 have been identified in various plants that have IR kinase-activating abilities similar to mcIRBP-19 that has a proved blood glucose-lowering activity that exhibits IR-binding potentials (Lo *et al.*, 2016).

Alpha-glucosidase and dipeptidyl peptidase IV (DPP-IV) are enzymes that play a significant role in the development of type 2 diabetes (T2D). Some studies have shown that dietary proteins—especially milk proteins—could be a natural source of these enzymes. The beneficial effects of these inhibitory peptides can be explained through several mechanisms, such as the satiety response, regulation of incretin hormones, insulinemia levels, and reducing the activity of carbohydrate degrading digestive enzymes (Patil *et al.*, 2015).

The extraction of peptides from egg yolk with a non-commercial enzyme obtained from Asian pumpkin has been described. These peptides remaining after phospholipid removal, and their four synthetic analogs were investigated. One of them, with the sequence LAPSLPGKPKPD, exhibited α-glucosidase inhibitory activities, related to their antidiabetic (α-glucosidase and DPP-IV inhibitory activities) properties. This peptide also showed the highest antioxidant activity as a free radical scavenger (Zambrowicz *et al.*, 2015).

Soy protein is of particular interest because can be utilized as an aid to treat MS. Its relation to several health benefits, including weight loss and prevention of complications related to cardiovascular risk factors, as well as the improvement of lipid profile and glucose and insulin homeostasis has been documented (Velasquez and Bhathena, 2007).

Animal models have demonstrated that soy protein has a positive effect on weight and fat loss that was especially prominent when comparing casein-based diets to soy-based foods. Some studies suggested that β-conglycinin fraction is responsible for the effect of soy protein in weight and fat loss (Song *et al.*, 2008; Moriyama *et al.*, 2014).

Applications and Production in Food Industry

Nutraceutical

Nutraceuticals are substances of natural origin that can be extracted from various sources (e.g. fruits, plants, lignocellulosic biomass, and algae) and that have important health benefits when incorporated into food or pharmaceutical formulations. In recent years, functional

foods and nutraceuticals have attracted much attention, particularly for their impact on human health and prevention of certain diseases (Meisel, 1997; Lee *et al*., 2013).

Considering that most functional peptides are present in complex matrices containing a large number of hydrolyzed protein fractions, their separation and purification are required. Conventional pressure-driven processes can be used for amino acids and peptides separation but are limited by their fouling problems and their low selectivity when separating similar sized biomolecules (Bazinet and Firdaous, 2009).

Processes combining an electrical field as a driving force and porous membranes have been developed for the separation of biopeptides to obtain better-purified products. More recently, electrodialysis using ultrafiltration membranes has been developed to fractionate simultaneously acidic and basic peptides using a conventional electrodialysis cell, in which some ion exchange membranes are replaced by ultrafiltration ones (Bazinet and Firdaous, 2013).

After separation and identification of BP in three hypoallergenic infant milk formulas, the identity of 24, 30, and 38 BP was confirmed in each of the three infant milk formulas. A significant number of these peptides were reported as inhibitors of ACE. However, the presence of sequences with other biological activities such as antihypertensive, anti-thrombotic, hypocholesterolemic, immunomodulation, cytotoxicity, antioxidant, antimicrobial, antigenic, or opioid was also confirmed (Catala-Clariana *et al*., 2013).

Functional ingredients

Peptides derived from the milk of cow, goat, sheep, buffalo, and camel exert multifunctional properties on human health. Additionally, medicinal plants are a rich source of natural antioxidants that are increasingly used in food manufacturing, because they provide valuable nutritional and therapeutic properties and retard oxidative degradation of lipids thus improving, the quality and nutritional values of foods regarded as functional (Shori and Baba, 2014). For instance, the effects of *Allium sativum* on total phenolic content, proteolysis by o-phthaldialdehyde assay, antioxidant activity by radical inhibition and capacity to inhibit α-amylase and α-glucosidase activities *in vitro* were higher in camel milk yogurt (MY) than cow MY (Amal and Ahmad, 2014).

The tendency to confer new functional properties to fermented dairy products by supplementation with BP to develop health-promoting foods is steadily increasing (Hafeez *et al*., 2013). One approach exploits the proteolytic system of LAB or food grade enzymes, or the combination of both, to release the functional peptides from the milk proteins directly in the fermented milk products. In another strategy, the BP are obtained outside of the product through the hydrolysis of the purified proteins by the same enzyme sources. Finally, in the last procedure the BP, initially identified from the milk proteins, are produced by microorganisms using recombinant DNA technology (Hafeez *et al*., 2014).

Different strategies that can be employed to enhance the production of BP from milk proteins that will be eventually used to functionalize fermented dairy products. One approach exploits the proteolytic system of LAB or food grade enzymes or the combination of both to release the functional peptides from the milk proteins directly in the fermented milk products. In another strategy, the BP are obtained outside of the product through the hydrolysis of the purified proteins by the same enzyme sources. Finally, in the last procedure the BP, initially identified from the milk proteins, are produced by microorganisms using recombinant DNA technology (Hafeez *et al*., 2014).

Several BP, mainly potential antihypertensive peptides from enzyme-modified cheese prepared by commercial and *Lactobacillus casei* enzymes, were purified and identified. Enzyme modified cheese samples were prepared by the combination of Neutrase®, *L. casei* enzymes, and Debitrase. The presence of sites containing potential antihypertensive peptides suggests that the purified peptides may have these properties. Thus, the enzyme-modified cheese process, mainly designed to produce flavour ingredients, may simultaneously produce BP, which are considered to be of physiological importance (Haileselassie *et al*., 1999). Such BP may find use in the treatment of diarrhoea, hypertension, thrombosis, dental carries, oxidative stress, mineral malabsorption, and immunodeficiency. These BP may be employed in the formulation of functional foods, nutraceuticals, and drugs for health improvement (Haque *et al*., 2008).

Alpha-lactalbumin (α-La) is a globular protein found in all mammalian milk that can be utilized in the production of functional foods. It has been used as an ingredient in infant formulas (Ambika, 2010). α-La has been recognized as a source of peptides with antitumour and apoptosis, anti-ulcerative, immune modulating, antimicrobial, antiviral, antihypertensive, opioid, mineral binding, and antioxidative activities. BP are released from α-La during the fermentation or ripening of dairy products by starter and non-starter microorganisms and during digestion by gastric enzymes. BP are also produced by deliberate hydrolysis of α-La using animal, microbial, or plant proteases (Kamau *et al*., 2010).

Inflammatory bowel diseases (IBD) are disorders affecting the GI tract, with prejudicial effects on the quality of life. These diseases are caused by different factors including dietary habits, and the symptoms include mucosal inflammation increased intestinal permeability and immune system dysfunction. The influence of the western diet, obesity, and various nutraceuticals/functional foods (BP, phytochemicals, omega 3-polyunsaturated fatty acids, vitamin D, probiotics, and prebiotics) have an influence on the course of IBD and provide some hints that could be useful for nutritional guidance (Uranga *et al*., 2016).

Production

There is an increasing commercial interest in the production of BP from various sources (Figure 1). Industrial-scale production of such peptides is, however, hampered by the lack of suitable technologies which retain or even enhance the activity of BP in food systems (Korhonen and Pihlanto, 2003; Korhonen and Pihlanto, 2006; Pihlanto, 2006; Kamau *et al*., 2010).

BP can be produced from milk proteins through fermentation of milk. In particular, antihypertensive peptides have been identified in fermented milk, whey and ripened cheese. A few of these peptides have been commercialized in the form of fermented milks (Korhonen and Pihlanto, 2003).

On the other hand, some BP have been identified in fermented dairy products, and there are already a few commercial dairy products enriched with blood pressure-reducing milk protein peptides. However, there still is a need to develop methods to optimize the activity of BP in food systems and to enable their optimum utilization in the body (Korhonen and Pihlanto, 2007).

The separation and purification of BP involving the development of automated and continuous systems is an important field for food chemists. Much effort has been devoted to developing selective column chromatography methods that can replace batch methods of salting out or using solvent extraction to isolate and purify BP. These developments will allow the recovery of BP with minimal destruction

to guaranty their incorporation into functional foods or for specific nutraceutical applications (Kitts and Weiler, 2003).

Enzymatic hydrolysis

Enzymatic digestion is the most efficient and reliable method to produce peptides with target functionalities, including antioxidant activity. A broad range of antioxidant peptides and peptide mixtures (hydrolysates) have been produced from soy, corn, potato, peanut, milk, whey, egg, and meat proteins. The antioxidant efficacy of protein hydrolysates and peptides depends on the source of proteins, the protein substrate pretreatment, the type of proteases used, and the hydrolysis conditions applied. Both pure and crude enzymes can be used to produce antioxidative peptides. However, to reduce the production cost, crude protein mixtures are preferred (Zarei *et al.*, 2012).

BP can be obtained from milk proteins in fermented products by proteases of LAB. For instance, BP can be produced from bovine caseins by growing *Streptococcus thermophilus* 4F44 (247 peptides identified, 143 were derived from β-casein) (Chang *et al.*, 2014).

The enzymatic hydrolysis of β-lactoglobulin and the fractionation of peptides were performed in one step in an electrodialysis cell with ultrafiltration membranes stacked, 15 anionic and 4 cationic peptides were detected in the anionic and cationic peptide recovery compartments. Amongst these 15 anionic peptides, 2 hypocholesterolemic, 3 antihypertensive, and 1 antibacterial peptide were recovered (Doyen *et al.*, 2013).

BP with ACE inhibitory and antioxidant activity have been obtained from Thornback ray skin gelatin upon hydrolysis with two different proteases. Hydrolysates with protease from *Bacillus subtilis* A26 (TRGH-A26) displayed ACE inhibitory activity whereas Neutrase® hydrolysate from *Bacillus amyloliquefaciens* (TRGH-Neutrase) showed antioxidant activity (Lassoued *et al.*, 2015).

Five commercially available food-grade microbial protease preparations were evaluated for their ability to hydrolyse meat myofibrillar and connective tissue protein extracts. Fungal protease or HT proteolytic (HT) hydrolysed both meat protein extracts, producing peptide hydrolysates with significant *in vitro* antioxidant and ACE inhibitor activities. Gel permeation chromatography sub-fractionation of the crude protein hydrolysates showed that the smaller peptide fractions exhibited the highest antioxidant and ACE inhibitor activities. Cell-based assays indicated that the hydrolysates present no significant cytotoxicity towards Vero cells. The results indicate that HT protease hydrolysis of meat myofibrillar and connective tissue protein extracts produces BP that are non-cytotoxic, should be stable in the GI tract and may contain novel BP sequences and hydrolysates retained bioactivity after simulated GI hydrolysis challenge (Ryder, 2016).

Microbial fermentation

The production of antioxidant peptides by microbial fermentation rather than using purified enzymes is an integral part of healthy food production in many countries. Natto and tempeh are fermented soybean products that contain antioxidant peptides by the action of fungal proteases (Wongputtisin *et al.*, 2007). The type, amount, and activity of the peptides produced depend on the particular cultures used. Douchi, also a soybean product fermented by fungal cultures (e.g. *Aspergillus spp.*), contains antioxidant peptides released by microbial enzymes.

Fermentation of milk proteins using the proteolytic systems of LAB is an attractive approach for the generation of functional foods enriched in BP given the low cost and positive nutritional image associated with fermented milk drinks and yogurt (Hayes *et al.*, 2007).

A novel strategy to enhance ACE inhibitory activities of navy bean consist in the preparation of navy bean milk (NBM) which was then subjected to fermentation with *Lactobacillus bulgaricus*, *Lactobacillus helveticus* MB2-1, *Lactobacillus plantarum* B1-6, and *Lactobacillus plantarum* 70810. All fermented NBM showed higher ACE inhibitory activity compared to the unfermented ones, for which 2 hours, 3 hours, and 5 hours were found to be the optimum fermentation periods for respectively *Lactobacillus plantarum* 70810, *Lactobacillus plantarum* B1-6 and *L. bulgaricus*. The subsequent *in vitro* GI simulation of all fermented extracts reduced IC50 values and the extracts fermented by *Lactobacillus plantarum* B1-6 exerted the lowest IC50 value. The investigation contributed to gain knowledge to obtain probiotic products with potential to serve as functional ingredients to treat hypertension (Rui *et al.*, 2015).

Bifidobacterium bifidum MF 20/5 ferments milk and the product of fermentation possesses stronger ACE inhibitory activity than other LAB, including *L. helveticus* DSM 13137. A novel ACE-inhibitory peptide LVYPFP and other BP such as the ACE-inhibitor LPLP and the antioxidant VLPVPQK were identified in the fermented milk. B. bifidum released a larger amount of peptides than *L. helveticus* but no IPP or VPP were detected in *B. bifidum* fermented milk. Likewise lactotripeptide concentrations and ACE inhibition were higher in *L. helveticus* fermented milk when the pH was maintained at 4.6. This may represent a technical advantage for *B. bifidum* that reduces the pH at a slow enough rate to facilitate the peptide generation without the need for pH control (Gonzalez-Gonzalez *et al.*, 2013).

The fermentation characteristics, ACE-inhibitory activity, and contents of Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) peptides of stored yogurt (4°C for 28 days) fermented by *L. helveticus* isolate H9 from cow, mare, and soy milks have been evaluated. During storage, the pH and titratable acidity remained stable in yogurts produced from all milk types and all inoculation concentrations. The ACE-inhibitory tripeptides VPP and IPP as determined by ultra-performance liquid chromatography-tandem mass spectrometry were not produced in yogurt made from soy milk or mare milk. These evaluations indicate that *L. helveticus* H9 has good probiotic properties and would be a promising candidate for the production of fermented food with probiotic properties (Wang *et al.*, 2015).

Chemical synthesis

Chemical organic synthesis is an indispensable tool to obtain organic molecules displaying particular physicochemical properties. The use of *in silico* protocols (Kishore, 2004; Dziuba and Dziuba, 2010; Udenigwe *et al.*, 2013), as well as established experimental approaches, in solution or solid phase (Saladino, 2012), allow the design and construction of molecules with various molecular complexities that can be used for biological studies (Smacchi and Gobbetti, 2000; Freidinger, 2003; Rajesh and Iqbal, 2006).

The therapeutic roles exhibited by many BP have elicited the interest to obtain them by chemical synthesis to treat certain pathological conditions related to oxidation (Ialenti *et al.*, 2001; Van Lancker *et al.*, 2011). Oral administration of a chemically synthesized peptide (Lys-Arg-Glu-Ser) lowered LDL peroxidation, alleviated inflammation, and reduced atherosclerosis in apoE-null mice (Navab *et al.*, 2004). Interestingly, changing the order of the peptide sequence to Lys-Glu-Arg-Ser resulted in the loss of all biological activity, suggesting a particular structure-activity relationship. Larger peptides that are synthesized to contain amphipathic helixes can also function to

suppress LDL peroxidation and reduce inflammation (Navab *et al.*, 2009).

Peptides containing the active Pro-His-His fragment have been synthesized, and all showed remarkable inhibition of lipid peroxidation. A series of synthesized histidine-containing peptides with sequences present in human paraoxonase 1 (an enzyme associated with HDL) strongly inhibit oxidation of lipoproteins (Nguyen *et al.*, 2009). The antioxidant actions of these peptides were attributed to their metal-ion chelation and radical-scavenging capabilities based on the Cu 2+ binding and peroxyl radical-quenching tests. The presence of tyrosine and cysteine residues was essential for the elicitation of the antioxidant activity. A synthetic histidine-containing the analog of the human albumin *N*-terminus fragment Asp-Ala-His-Lys is also highly effective in inhibiting the Cu 2+ induced formation of ROS (Bar-Or *et al.*, 2001).

Among an antioxidant tripeptide library, the tripeptides, Tyr-His-Tyr and Pro-His-His are especially effective in stabilizing radical and non-radical oxygen species, including peroxynitrite and lipid peroxide (Saito *et al.*, 2003).

In recent years, there has been a growing interest in de novo design and construction of novel synthetic peptides that mimic protein secondary structures to develop potent peptide analogs and peptidomimetics displaying unique pharmaceutical properties (Kishore, 2004). BP are considered the new generation of biologically active regulators (Lemes *et al.*, 2016) not only to prevent oxidation and microbial degradation of foods but also to enhanced the treatment of various diseases and disorders.

Some emerging technologies to recover BP from residual waste (Harnedy and Fitzgerald, 2012) and to transform them into added-value products, as well as to facilitate large-scale recovery (Kitts and Weiler, 2003; Agyei and Danquah, 2011) and purification of peptides aiming at future applications for the pharmaceutical (Agyei and Danquah, 2011) and food industries (Korhonen and Pihlanto, 2007) are currently under investigation.

Progress in liquid chromatography and mass spectrometry technologies offers a great opportunity for the identification of BP. However, in many cases the direct application of this technology does not allow the detection of the peptides due to signal suppression.

Processes combining an electrical field as the driving force to porous membranes have been developed for the separation of bioactive peptides to obtain better purified products. More recently, electrodialysis with ultrafiltration membranes has been developed to fractionate simultaneously acidic and basic peptides, using a conventional electrodialysis cell, in which some ion exchange membranes are replaced by ultrafiltration ones (Bazinet and Firdaous, 2009).

Nanofiltration (NF), pressure-driven process, and electrodialysis with ultrafiltration membranes (EDUF) (electrically-driven process) were compared in terms of mass flux and mass balance. The two processes lead to different results since NF was more efficient in terms of mass flux than EDUF when compared on a same basis, while EDUF recovered larger range of peptide MWs and amount of polar amino acids. The antioxidant capacity of the fractions was analysed and the more relevant fractions were tested for their potential neurone cells protection against ROS. The peptides isolated by EDUF in the anionic recovery compartments showed an increase of the antioxidant capacities. These showed that coupling NF and EDUF in a same process line would optimize their own separation performances and allow the production of more specific peptide fractions than alone (Langevin *et al.*, 2012).

Multifunctional properties of several antimicrobial milk peptides have been exhibit an immune defence against several microbial infections. Whey constitutes five major proteins, such as α-lactalbumin, glycomacropeptide, β-lactoglobulin, protease peptone, immunoglobulins, and serum albumin, that together make up 85% of whey protein, whereas casein contains αs1-casein, αs2-casein, β-casein, and κ-casein and have been proven that these peptides are the potent inhibitors of pathogenic organisms such as bacteria e.g. *Escherichia*, *Helicobacter*, *Listeria*, *Salmonella*, and *Staphylococcus*; yeast, and filamentous fungi (Mohanty *et al.*, 2016).

Ultrasound is a novel, robust, green, and rapid technology suitable for scale up, can enhance the efficiency of protein digestion, extraction, production and drug delivery of BP, this principally acts by generating bubble cavitation in the biological matrix. It has been extensively reported for extraction of proteins and peptides from natural products facilitating higher yields and rates of extraction. Ultrasound assisted encapsulation of peptide based drugs with biodegradable polymers can improve stability and bioavailability. Moreover, in sonophoresis applications, low-frequency ultrasound can be used to transport high-MW peptide drugs (Kadam *et al.*, 2015).

Conclusions

Hidden between the chemical structure of proteins present in food matrixes, and other natural sources, lie an immense amount of BP. Enzymatic hydrolysis occurring during digestion or fermentation can liberate an enormous amount of BP whose activities span from antimicrobial, anti-thrombotic, antihypertensive, opioid, immunomodulatory, mineral binding, and antioxidative. As a result of this broad spectrum of activities, BP have the potential to be used as food additives and ingredients of pharmaceuticals for the treatment or prevention of some medical conditions and life style diseases, such as obesity, diabetes type II and hypertension.

Despite the significant progress in the isolation and purification of BP from several natural sources, as well as the assessment of their bioactivities, there still are several obstacles to overcome, particularly from the technological viewpoint to produce them at large scale without losing activity. The increasing interest of the scientific community in the identification, purification, chemical synthesis and uses of BP and the food industry to use BP in commercial products will contribute to improving human health.

Conflict of interest statement. None declared.

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