Bioactive peptides: A review
Adrián Sánchez* and Alfredo Vázquez**

*Laboratorio de Investigación en Inmunquímica, Dr. Márquez 162, Cuauhtémoc, Doctores, 06720, CDMX and **Departamento de Química Orgánica, Facultad de Química, Universidad Nacional Autónoma de México, México

Correspondence to: Alfredo Vázquez, Departamento de Química Orgánica, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, CDMX, México. E-mail: joseavm@unam.mx

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; Möller 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 (Möller 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 Klok, 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 antioxidant activity (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 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 hirudin, 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 anti-cancer 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, neuramidase, 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, HLPLLHR, HGVLQ, GLYSPH, LVRVQ, YLSE, DQVPR, LPLL, and VKPVAPE, 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 hydrolysates 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; Jäskälä 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 carbonyl-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.

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

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 casem 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 (Bourtou 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.
(Harnedy and Fitzgerald, 2012; 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), antiancides, immunostimulatory, calcium-binding, hypcholesteremic 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-pircryldrazyl 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-His were obtained from sardine (Sardinella aurita) industrial wastes generated using crude enzyme extract from sardine (Sardinella pilchardus). The first tripeptide displayed...
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>
<thead>
<tr>
<th>Peptide</th>
<th>Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celenamide B</td>
<td>Nazunamide A</td>
</tr>
<tr>
<td>Jasplakinolide</td>
<td></td>
</tr>
<tr>
<td>Cyclic depsipeptide isolated from the marine sponge <em>Jaspis johnstoni</em>, induces apoptosis associated with caspase-3 activation and a decrease in Bcl-2 protein expression. Zhen et al. (2011).</td>
<td></td>
</tr>
<tr>
<td>Dolastin</td>
<td></td>
</tr>
<tr>
<td>Peptide isolated from the mollusk <em>Dolabella auricularia</em>. Anticancer activity against the P388 cell line; inhibits tubulin polymerization and tubulin dependent GTP hydrolysis. Aneiros (2004).</td>
<td></td>
</tr>
<tr>
<td>Trisindoline</td>
<td></td>
</tr>
<tr>
<td>Peptide from the marine sponge <em>Hyrtios altum</em>. Shows antibacterial activity against <em>Escherichia coli</em>, <em>Bacillus subtilis</em> and <em>S. aureus</em>. Kobayashi (1994).</td>
<td></td>
</tr>
<tr>
<td>Mutoporin</td>
<td></td>
</tr>
<tr>
<td>Radiosumine</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Examples of peptides with known activity.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Origin</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSDKKIAK</td>
<td>Antimicrobial</td>
<td>Capriotti et al. (2016)</td>
<td></td>
</tr>
<tr>
<td>EQLTK</td>
<td>Antimicrobial</td>
<td>Wada and Lonnerdal (2014)</td>
<td></td>
</tr>
<tr>
<td>LKP</td>
<td>Antihypertensive, ACE</td>
<td>Erdmann et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>AKSY</td>
<td>Antihypertensive, ACE</td>
<td>Harney and Fitzgerald (2011)</td>
<td></td>
</tr>
<tr>
<td>KROKYD</td>
<td>Antihypertensive, ACE</td>
<td>Lafarga (2014)</td>
<td></td>
</tr>
<tr>
<td>KRPHPKH</td>
<td>Antihypertensive, ACE</td>
<td>El-Salam (2013)</td>
<td></td>
</tr>
<tr>
<td>SVPQPK</td>
<td>Antihypertensive, ACE</td>
<td>Hernández-Ledesma et al. (2007)</td>
<td></td>
</tr>
<tr>
<td>VVPPWYQ</td>
<td>Antihypertensive, ACE</td>
<td>Lee et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>VECYGPNPQF</td>
<td>Antioxidant</td>
<td>Ricci-Cabello et al. (2016)</td>
<td></td>
</tr>
<tr>
<td>WYSALMAASDI</td>
<td>Antioxidant, radical scavenging</td>
<td>Capriotti (2015)</td>
<td></td>
</tr>
<tr>
<td>IVNPHDHQNLK</td>
<td>Antioxidant</td>
<td>Nongonierma and Fitzgerald (2015)</td>
<td></td>
</tr>
<tr>
<td>WYSALMAASDI</td>
<td>Antioxidant</td>
<td>Khat et al. (2015b)</td>
<td></td>
</tr>
<tr>
<td>KREGTGY</td>
<td>Antihypertensive, ACE</td>
<td>Gurin et al. (2016)</td>
<td></td>
</tr>
<tr>
<td>VPPPP</td>
<td>Antihypertensive, ACE</td>
<td>Patil (2015)</td>
<td></td>
</tr>
<tr>
<td>IBYW</td>
<td>Hipoglucoamiant</td>
<td>Aygei et al. (2016)</td>
<td></td>
</tr>
<tr>
<td>KLPGF</td>
<td>Hipoglucoamiant</td>
<td>Kaman (2010)</td>
<td></td>
</tr>
<tr>
<td>GGGGYMPYLR</td>
<td>Immunomodulatory</td>
<td>Sanjukta and Rai (2016)</td>
<td></td>
</tr>
<tr>
<td>GLF</td>
<td>Anticancer</td>
<td>Messe (1999)</td>
<td></td>
</tr>
<tr>
<td>GLDVDLL</td>
<td>Anti-inflammatory</td>
<td>Fan (2014)</td>
<td></td>
</tr>
<tr>
<td>YGLF</td>
<td>Platelet activating inhibition</td>
<td>Fan (2014)</td>
<td></td>
</tr>
</tbody>
</table>

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, 2008), potato protein (Wang and Xiong, 2005) (Pihlanto et al., 2007), 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$_2^-$) 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), *Opistophtalma carinatus* (opistho-porin 1 and 2), *Parabuthus schlechteri* (parabuto-porin), and *Randinus imperator* (pandinin 1 and 2). From Hymenoptera venom, *Opisthacanthus madagascariensis* (parabuto-porin) and *Parabuthus schlechteri* (opisto-porin 1 and 2), *Hadrurus aztecus* (hadrurin), *Opisthacanthus madagascariensis* (parabuto-porin) and *Parabuthus schlechteri* (opisto-porin 1 and 2), *Hadrurus aztecus* (hadrurin), and *Parabuthus schlechteri* (opisto-porin 1 and 2), *Hadrurus aztecus* (hadrurin), and *Parabuthus schlechteri* (opisto-porin 1 and 2).

Peptides derived from 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 Myceolpeptonoine saichiiomini, 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-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 allergic 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-NH2), an endogenous opioid peptide that exhibits high selectivity for MOR, such as nociception, 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 LKKIQRYQKFLPQY; VYQHQKAMKFWQKTKPVYPYRY; VYQHQKAMKFWQKTPKVYPYRYL; AMKFWQKTKPVYPYRYL; KFWQKTPKVYPYRYL. They have been isolated from peptic hydrolysates 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, insulin resistance, 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 (Velásquez and Bhatena, 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 Firdaus, 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 Firdaus, 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, hypcholesterolemic, 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 α-phthalaldehyde assay, antioxidant activity by radical inhibition and capacity to inhibit α-amylase and α-glucosidase activities in vitro were higher in camel milk yoghurt (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 Debristrase. 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 anti-tumour and apoptosis, anti-atherosclerotic, immune modulating, anti-microbial, 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 cytototoxicity 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 205 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 (Saladin, 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 (Lalenti 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-Arg-Glu-Ser resulted in the loss of all biological activity, suggesting a particular structure-activity relationship. Larger peptides that are synthesized to contain amphipathic helices 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; Ag eye and Danquah, 2011) and purification of peptides aiming at future applications for the pharmaceutical (Ag eye 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 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 (Bazzinet 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 peptide, 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 matrices, 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.

References

A. Sánchez and A. Vazquez, 2017, Vol. 1, No. 1

Coughtry B: Analytical Technologies in the Biomedical and Life Sciences, 803: 41–53.


