



## A review on bioactive peptides derived from meat and by-products: Extraction methods, biological activities, applications and limitations

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### ABSTRACT

Meat and its by-products offer a rich source of bioactive compounds which have potential applications in both the food and pharmaceutical industries. In this review, we present several extraction methods and report the identification and properties of bioactive peptides. We also examine the challenges and limitations associated with their use in food applications. Enzymatic hydrolysis and fermentation using starter cultures are common methods for generating bioactive peptides from meat proteins. Additionally, natural gastrointestinal digestion can also produce bioactive peptides. However, emerging technologies like high hydrostatic pressure, subcritical extraction and pulsed electric fields can improve hydrolysis and increase the yield of bioactive peptides. Online bioinformatics applications have emerged as an established method for identifying potentially bioactive peptides. These tools reduce the cost and time required for traditional methods of research. Finally, incorporating bioactive peptides into diets for specific purposes such as supporting vulnerable populations like children and the elderly ensures safety and efficacy.

### 1. Introduction

Meat is a highly valued livestock product, often preferred by many customers as their primary source of animal protein. Additionally, the meat industry, like many other agri-food sectors, produces valuable by-products. However, if not effectively managed, the production of by-products can give rise to significant economic and environmental challenges for the industry. In the meat industry, the process of slaughtering animals generates not only the desired end-products but also different animal by-products. These animal by-products encompass entire bodies or specific parts of animals, as well as other animal-derived products which are not intended for direct human consumption (Regulation (EC) No 1069/2009, 2009). Proper management and disposal of these by-products are crucial to mitigate the potential adverse impacts on both the industry and the environment.

The importance of these by-products varies depending on factors such as tradition, culture and religion. Some of the most significant ones include remains, skins, bones, meat trimmings, blood, fatty tissues,

horns, feet, hoofs and internal organs. Several industrial technologies are currently being applied to treat and reduce by-product production because the majority of these by-products are still either discarded as waste or used for low-value (Di Bernardini et al., 2011; Toldrá, Aristoy, Mora, & Reig, 2012). However, meat by-products could be used as essential raw materials for producing high-value-added components like bioactive peptides for the functional foods market, which is currently in high demand (Borrajo et al., 2019; Chernukha, Fedulova, & Kotenkova, 2015). They are also rich in lipids, carbohydrates, fiber, vitamins and proteins resulting in versatility for a wide range of applications within the food industry and pharmacy (Ahmed & Muguruma, 2010; Bechaux, Gatellier, Le Page, Drillet, & Sante-Lhoutellier, 2019). Therefore, proper management and utilization of meat by-products could bring significant benefits to the meat industry and environment.

Bioactive peptides are short polymers consisting of 2 to 20 amino acids with low molecular weight in contrast to proteins. They could be produced through processes like enzymatic hydrolysis, fermentation or other emerging technologies. These peptides might exhibit biological

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activity and could offer several health benefits (as depicted in Fig. 1). In recent years, there have been publications on the production and characterization of bioactive peptides with antioxidant, anti-hypertensive, anti-inflammatory and antimicrobial properties as can be checked in the following paper (Rezaharsanto & Subroto, 2019). This review highlights the characterization, extraction, potential health benefits and possible applications of bioactive peptides derived from various meat sources and their by-products.

On the other hand, the absorption and bioavailability in both *in vitro* and *in vivo* experiments should be also considered. The interaction of biopeptides with the food matrix could affect their activity, leading to depletion or slow release during gastrointestinal digestion (Sun, Acquah, Aluko, & Udenigwe, 2020; Udenigwe & Fogliano, 2017). To address this issue, several techniques have been proposed such as incorporating biopeptides into fiber-rich foods, using mild food processing techniques and employing encapsulation, conjugation nanocarriers, and permeation enhancers to improve their intestinal absorption. The Scopus database was used to retrieve documents published between 2014 and 2023, using the keywords “bioactive peptides” and “meat” in the abstracts and titles, resulting in a total of 369 documents. Additionally, combinations of keywords including “bioactive peptides”, “meat sources”, “meat by-products”, “extraction”, “purification”, “identification”, “bioactivities”, “health impacts”, “pharmaceutical applications”, “food applications” and “bioavailability” were also used to expand the search for more papers. Fig. 2 displays the main keywords found in the 369 scientific documents, according to VOSviewer software (<https://www.vosviewer.com/> accessed on 27/10/2022). The bibliographic analysis revealed that most of the studies are focused on by-products of pig and cattle (green cluster), as well as research in the bioactivity of peptides. These bibliometric indicators highlight the significance of exploring the bioactive peptides present in meat by-products for their potential health benefits and various applications in the food and pharmaceutical industries.

## 2. Searching bioactive peptides across a variety of meat sources

Meat is an essential source of protein and nutrients for human nutrition, with the most commonly consumed species being pigs and cattle from livestock (Hoffman & Cawthorn, 2013). Along with water, protein and fats, meat also contains minerals, carbohydrates, vitamins and other bioactive components in smaller amounts (Lafarga & Hayes, 2014; Wood, 2017). In vertebrates, there are three types of muscle: skeletal, cardiac and smooth. The fundamental unit of the structure of muscles is the myofiber which is a lengthy, multinucleated muscle cell. These cells are organized in bundles of fibers joined by thin sheets of

connective tissue called perimysium. Meat fibers contain myofibrils, which are contractile structures made up of dense (myosin) and slim (actin) protein filaments (Hill & Olson, 2012). These myofibrils are responsible for the texture and flavour of meat products, providing essential nutrients in a healthy diet.

Furthermore, meat from different species possesses distinct properties due to variations in its chemical composition. Bovine, porcine and ovine contain a range of skeletal muscle proteins that differ in terms of their molecular size and isoelectric point. An *in silico* study of the meat proteome has reported that meat could have various health-promoting benefits, including antioxidant, anti-thrombotic, anticancer, immunomodulatory and antimicrobial capacities as well as producing particularly anti-hypertensive activity (Udenigwe & Howard, 2013). Other *in silico* studies suggested that some biopeptides from pork could be useful in the regulation of diabetes type 2, due to mainly the large contribution of amino acids phenylalanine and tryptophan (Keška, Stadnik, Bak, & Borowski, 2019). Supporting this study, Martini, Conte, and Tagliacozchi (2019) reported peptides from pork as a good source of dipeptidyl peptidase IV (DPPV-IV) inhibitors. During the cooking process and gastrointestinal digestion beef and pork released bioactive peptides, previously identified in other food matrices. Specifically, those peptides derived from muscle in pork have been found to inhibit the Angiotensin I-converting enzyme (ACE).

### 2.1. Peptides from meat by-products

The meat industry is generating a huge number of valuable by-products (e.g., skins, bones, blood, collagen and offal), which could be used as a source of bioactive peptides (Mora, Gallego, & Toldrá, 2018) adding value to the products and reducing their environmental impact in line with sustainability. For instance, blood is an excellent source of proteins, with haemoglobin being the most abundant protein resulting in a rich source of iron and it can be hydrolysed to produce bioactive peptides such as neokytorphin ( $\alpha$ 137–141), which has strong antimicrobial properties (Abou-Diab et al., 2020). In line with this, an *in vitro* study has shown that different hydrolysates of plasma from deer, sheep and pork using plant (papain and bromelain) and fungal (FP400 and FPII) proteases showed antioxidant properties (Bah, Bekhit, Carne, & McConnell, 2015). On the other hand, collagen is an abundant meat industrial by-product, because of is the primary component of the bones, teeth, horns and skin. Despite being considered a low-quality protein, it could be hydrolysed to produce bioactive peptides with various biological functions (Jayathilakan, Sultana, Radhakrishna, & Bawa, 2012; Toldrá, Mora, & Reig, 2016). These by-products offer a promising source of biopeptides, which could be utilized in several industrial sectors. For

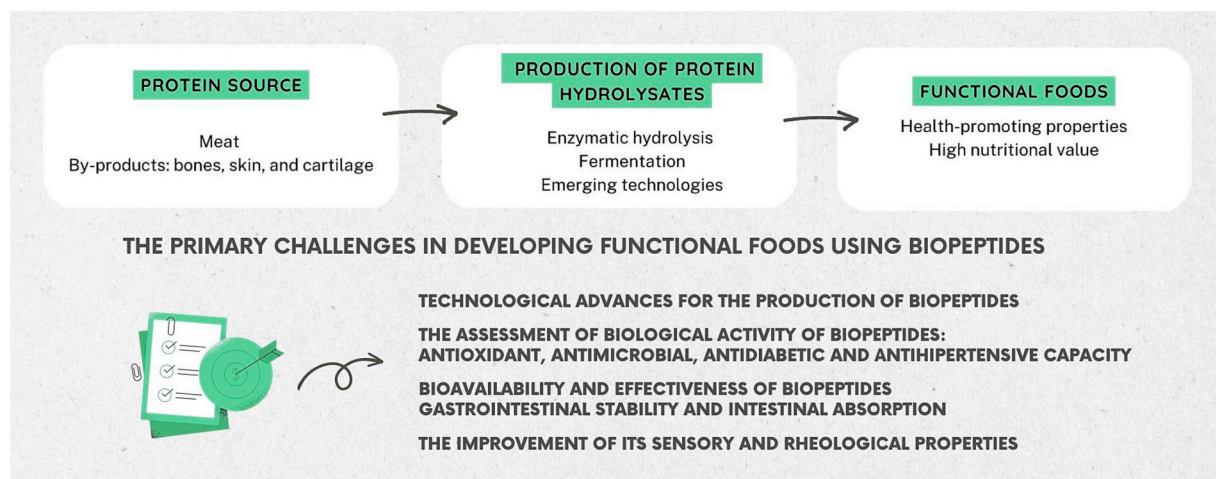


Fig. 1. Primary challenges in the development of novel foods using biopeptides as functional ingredients.



**Table 1**  
Several examples of harnessing meat by-products for producing bioactive peptides.

Meat by-product	Hydrolysis procedure	Health promoting effects of hydrolysates	Reference
Beef skeletal muscles	Enzymatic digestion: pepsin	Angiotensin-converting enzyme inhibitory activity Antimicrobial effects	(Maky & Zendo, 2021)
Collagen by products (beef and pork)	Acid hydrolysis under heating	Antioxidant and hypoglycemic activities	(Gaspardi et al., 2022)
Pig aorta tissues	Fine grinding	Against cardiovascular disorders	(Chermukha et al., 2015)
Deer, sheep and pig blood	Enzymatic hydrolysis: plant (papain and bromelain) and fungal (FP400 and FPII) enzymes	Antioxidant activity	(Bah et al., 2015)
Porcine haemoglobin	Sub-critical water hydrolysis	Antioxidant activity	(Álvarez, Tiwari, Rendueles, & Díaz, 2016)
Porcine blood plasma	Enzymatic hydrolysis	Antioxidant activity	(Liu, Kong, et al., 2010)
Porcine liver	Enzymatic hydrolysis: Alcalase, bromelain, flavourzyme and papain	Antioxidant activity	(López-Pedrouso, Borrajo, Pateiro, Lorenzo, & Franco, 2020)

endogenous enzymes present within muscle cells can contribute to high proteolytic converting proteins into peptides *in vivo*. After extraction, it is often necessary to fractionate and purify the peptide mixture. To achieve this ultrafiltration, gel filtration chromatography (GFC), Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC), size exclusion chromatography (SEC), and ion exchange chromatography (IEC) could be employed.

### 3.1.1. Enzymatic hydrolysis to obtain meat hydrolysates

Enzymatic hydrolysis is the most widely used method for producing peptides, particularly in large-scale industrial processes (Agyei & Danquah, 2011). The efficiency of this process is influenced by several variables such as pH, temperature, enzyme/substrate ratio, length of hydrolysis and enzyme deactivation. By modifying these factors, hydrolysis can be precisely controlled. For instance, for hydrolyzing animal muscle from bovine, porcine or deer, as well as porcine blood, pepsin and papain enzymes are commonly preferred. On the contrary, industrial food-grade proteinases such as Alcalase, Flavourzyme and Protamex are typically used to produce antioxidant peptides from pork blood plasma (Liu, Xing, Fu, Zhou, & Zhang, 2016). Alcalase was used to prepare porcine plasma protein hydrolysates and the resulting peptide HNGN showed a high level of antioxidant activity (Liu, Kong, Xiong, & Xia, 2010). Similarly, haemoglobin hydrolysates obtained from porcine plasma using Alcalase and Flavourzyme yielded a fraction of higher molecular weight with stronger antioxidant activity (Chang, Wu, & Chiang, 2007).

### 3.1.2. Microbial fermentation to obtain meat hydrolysates

Biopeptides can also be produced commercially through microbial fermentation. As microorganisms require proteins as a source of nitrogen, so extracellular proteins are broken down, releasing proteases that break down large peptides into smaller peptides and even free amino acids (Razzaq et al., 2019). One method for producing bioactive peptides through protein hydrolysis, particularly in dairy and vegetable foods, is based on the use of lactic acid bacteria (LAB). Bioactive

peptides can obtain from alpha and beta caseins, albumin and globulin from milk as well as rubisco from spinach, and beta-conglycinin for soy or gluten from cereals. These peptides could be used to control oxidative stress, lower blood glucose and cholesterol levels, and improve cardiovascular function, among other health benefits (Pessione & Cirrincione, 2016). However, in the case of meat fresh products, the activity of LAB is not enough to achieve extensive fermentations, and there is limited research on bioactive peptides isolated from meat products (Borrajo et al., 2019). Despite this limitation, two types of LAB (*Latilactobacillus sakei* CRL1862 and *Latilactobacillus curvatus* CRL705) were tested on porcine skeletal muscle, resulting in the hydrolysis of sarcoplasmic proteins and the identification of the peptide FISNHAY that showed ACE inhibitory activity (Castellano, Aristoy, Sentandreu, Vignolo, & Toldrà, 2013). On the contrary dry-fermented sausages, incorporate LAB and sodium caseinate into the formulation resulting in intense proteolysis during the curing process and the production of bioactive peptides (Mora, Escudero, Aristoy, & Toldrà, 2015).

### 3.2. Exploring emerging processing technologies for biopeptide production

While biochemical hydrolysis is widely used for generating bioactive peptides until now, green technologies are being developed to achieve greater yield and lower costs at an industrial level. New technologies such as high hydrostatic pressure (HHP), subcritical extraction and pulsed electric field (PEF) have been developed to improve hydrolysis, aiming to obtain bioactive peptides. Even, combining biochemical hydrolysis processes with the abovementioned technologies could be a way to overcome the limitations of traditional approaches (Tadesse & Emire, 2020).

The HPP is a non-thermal technique in which isostatic pressure (usually 100–1000 MPa) is quickly applied to liquid or partially liquid-based products. Using HHP technology to generate bioactive peptides from a broad spectrum of food by-products has been proposed (Marciniak, Suwal, Naderi, Pouliot, & Doyen, 2018). The effect of HHP on the digestion of porcine blood with trypsin and pepsin can decrease the time of incubation although bioactivity was not assayed (Toldrà, Parés, Saguer, & Carretero, 2011). For the production of porcine haemoglobin peptides a combination of moderate temperature and medium pressure under a nitrogen stream was employed, achieving 82% of haemoglobin was broken into average-size antioxidant peptides of 3.2 kDa (Álvarez, Rendueles, & Díaz, 2012).

The PEF is a non-thermal procedure that has been widely used to inactivate microorganisms and enzymes in liquid and semi-liquid foods but also can be used to increase extraction in the food and nutraceutical industry. This way PEF technology could improve the susceptibility of a protein to enzymatic hydrolysis. Indeed,  $\beta$ -lactoglobulin treated with PEF improved biopeptide generation through the induction of active sites for nucleophilic enzymatic action (Mikhaylin, Boussetta, Vorobiev, & Bazinet, 2017).

Subcritical water hydrolysis is a relatively novel strategy for protein extraction. This process employs water in a subcritical state at temperatures and pressures close to its critical point, resulting in a lower water dielectric constant similar to organic solvents. This methodology was used to recover porcine haemoglobin peptides (Álvarez et al., 2012). Numerous techniques for the production of biopeptides are currently available, and many are undergoing testing to determine their efficacy and result.

## 4. Cutting-edge techniques used in the detection and identification of bioactive peptides

The identification of bioactive peptides is a complex process, which requires the use of various analytical techniques depicted in Fig. 3. Liquid Chromatography with tandem mass spectrometry (LC-MS/MS), electrospray ionization (ESI), and matrix-assisted laser desorption/ionization (MALDI) MS are the most used methods for protein/peptide

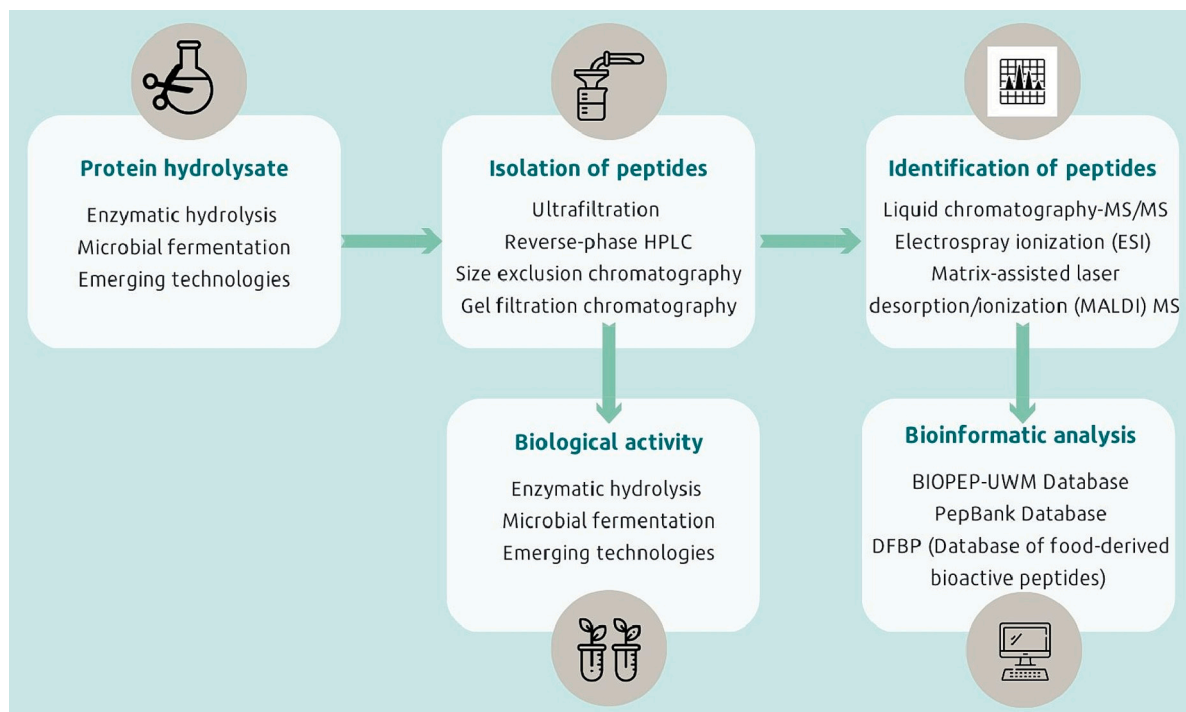


Fig. 3. Tools commonly used for analysis biopeptides from novel foods.

identification. These techniques provide valuable information about the presence and behaviour of bioactive peptides (Del Mar Contreras, López-Expósito, Hernández-Ledesma, Ramos, & Recio, 2008). Before the application of these methods, a purification step is required including ultrafiltration, size exclusion chromatography, gel filtration chromatography, ion exchange chromatography and high-performance liquid chromatography (Xing, Liu, Cao, Zhang, & Guanghong, 2019). Numerous scientific studies incorporate data from both *in vitro* and *in vivo* trials, along with *in silico* assessments. Online bioinformatics applications have emerged as established methods for researching and identifying potentially bioactive peptides, which could have significant implications.

Bioinformatics comprises low-cost and supplementary methods designed to identify potential bioactive peptides as a preliminary step before undertaking the time-consuming traditional methods such as *in vitro* and *in vivo* assays. These methods utilize several protein databases that contain data on the amino acid sequence of parents and peptides. Such information is valuable in choosing enzymes that can liberate peptides with predicted activities and characteristics. These databases may also be used to predict the physicochemical, functional and sensory attributes of peptides. The amino acid content and sequence of the parent protein can be used to infer the presence of bioactive peptides. Several tools, including UniProtKB and NCBI, are commonly employed to obtain such data. A wide range of databases and applications are available to carry out *in silico* research. For example, Minkiewicz et al. (2011) reported that BIOPEP is a relevant tool for estimating the antioxidant effects of bovine meat peptides, with collagen and elastin being the richest potential sources of peptides as well as the enzyme Proteinase K was found to be very suitable for the release of these peptides. PepBank is another innovative tool, which can be used to search peptide sequences in MEDLINE abstracts, offering valuable biological and medical information. This database is combined with ASPD, Uniprot and other curated peptide databases (Shtatland, Guettler, Kossodo, Pivovarov, & Weissleder, 2007). These bioinformatic applications have greatly facilitated the identification of potential bioactive peptides, reducing the cost and time involved in traditional methods. It should be noted that *in silico* studies predicting bioactivity, have limitations that

need to be acknowledged. Such studies often simulate complete protein hydrolysis of pure and linear protein sequences, ignoring the complex secondary and tertiary structures present in them. Therefore, while these results can provide an initial insight, they should be considered cautiously and further validated through additional experiments (Bechaux et al., 2019).

## 5. Bioactive properties of peptides obtained from meat and by-products

Diet has crucial implications for human health and there are many bioactive compounds, which play an important part in promoting health. Bioaccessibility and bioavailability are the most important factors in peptide bioactivity, but it is challenging to demonstrate the direct relationship between the biological activity of peptides using *in vitro* and/or *in vivo* assays. This is due to peptides must overcome proteolytic enzymes, gastrointestinal absorption, and blood protease hydrolysis, which could either inactivate them or enhance their physiological function. Certainly, peptides are receptors that attach to physiologic targets to conduct different signalling processes, and they have been shown to have regulatory activities that could contribute to positive health outcomes, largely through *in vitro* research, cell culture, and animal investigations. Numerous parameters, including molecular size, hydrophobicity, and charge, provided by the variation of amino acids in their structure, lead to a variety of peptides with a wide range of biological effects (Udenigwe & Fogliano, 2017). As we indicated physiologic functions of peptides released from meat and its by-products have been demonstrated to possess antioxidant antimicrobial, anti-diabetic, anti-hypertensive, and other properties (Fig. 1 and Table 1).

### 5.1. Antioxidant activity

Peptides with antioxidant activity are natural compounds with the capacity to prevent or slow down oxidative cell damage on oxidizable substrates. Hence, the primary benefit is their ability to protect the human body against oxidative stress, which can cause damage to cells and increase the risk of degenerative diseases (Liu et al., 2016).

Additionally, since antioxidants delay lipid oxidation and minimize rancidity without negatively affecting the nutritional or sensory characteristics, their action extends the shelf life and improves its quality (Kumar, Yadav, Ahmad, & Narsaiah, 2015). Peptides with antioxidant capacity can deactivate free radicals through two primary mechanisms: hydrogen atom transfer (HAT) and single electron transfer (SET) (Liu et al., 2016; Lorenzo et al., 2018). In this sense, it is desirable to use more than one antioxidant method for the search for the antioxidant activity of hydrolysates and/or peptides. To obtain a reliable estimation of their antioxidant activity, hydrolysates and/or peptides should be subjected to a HAT-based assay, one SET-based assay and one assay against primary or secondary lipid oxidation such as peroxide value or thiobarbituric acid reactive substances index value (Di Bernardini et al., 2012). The potential of dry-cured ham as a source of antioxidant peptides has been proved by Escudero, Mora, Fraser, Aristoy, and Toldrá (2013) who identified 27 antioxidant peptides through LC-MS/MS in Spanish dry-cured ham. The peptides SAGNPN showed the most radical scavenging activity, whereas GLAGA presented the higher reducing power. Functional hydrolysates obtained from meat by-products are becoming increasingly relevant due to their potent antioxidant activity and low production cost. Enzymatic digestion is a promising approach for generating these hydrolysates. Furthermore, meat by-products were assessed for generating antioxidant peptides. For instance, the porcine collagen hydrolysate produced by protease treatments from pork skin exhibited antioxidant capacity, highlighting the antioxidant peptide QGAR, that was synthesized for validation of antioxidant capacity (Li, Chen, Wang, Ji, & Wu, 2007). Concerning the liver, sarcoplasmic proteins isolated from bovine livers were hydrolyzed by thermolysin to separate, purify, and characterize antioxidant peptide fractions. After the use of several *in vitro* antioxidant assays (*i.e.*, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and  $\text{Fe}^{2+}$ ) authors reported that several fractions between 3 and 10 kDa showed antioxidant capacity (Di Bernardini et al., 2011). Moreover, the generation of peptides from porcine haemoglobin, using moderate temperature and medium pressure instead of enzymatic methods, resulted in low molecular weight peptides with antioxidant capacity (Álvarez et al., 2012). Another meat by-product tested was the water buffalo (*Cornu Bubali*) horn, where an aqueous fraction resulted in three antioxidant peptides (QYDQGV, YEDCTDCGN, and ADNANELFPPN) (Liu, Wang, Duan, Guo, & Tang, 2010).

### 5.2. Antimicrobial activity

The other important biological activity that is being investigated is the antimicrobial activity. In this regard, antimicrobial peptides (AMPs) are being developed for human consumption. They exhibit broad-spectrum antimicrobial activity against a variety of pathogens, including Gram-positive and Gram-negative bacteria, fungi, and parasites. The mechanisms of peptides to penetrate microbial membranes are different depending on the antimicrobial peptide mainly the barrel-stave mechanism, the toroid pore or wormhole mechanism and the carpet mechanism (Lafarga & Hayes, 2014). Beyond this, the AMPs can inhibit DNA and protein synthesis or kill target cells. To achieve this, the size, charge, hydrophobicity, amphipathicity and solubility are key properties of peptides' antimicrobial activities. AMPs must contain at least 7–8 amino acids to generate amphipathic molecules. This means that AMPs are regarded as lengthy peptides in comparison to ACE peptides (*e.g.*, 2–3 amino acids) (Bahar & Ren, 2013). Several studies were reported for haemoglobin molecule; this entire protein did not show antibacterial activity, but the fragments displayed an antimicrobial activity in human menstrual blood or the wound-healing process. Specifically, the bovine haemoglobin digested by pepsin exhibited antibacterial activity against *Micrococcus luteus* A270, *Listeria innocua*, *Escherichia coli*, and *Salmonella enteritidis*. Most fragments were identified in the  $\alpha$ -chain of haemoglobin and several fragments in the  $\beta$ -chain (Daoud et al., 2005; Naima Nedjar-Arroume et al., 2006; Naima Nedjar-Arroume et al., 2008; Hu et al.,

2011). Other examples have been found in the literature, such as four peptides, GFHI, DFHING, FHG, and GLSDGEWQ, derived from beef sarcoplasmic proteins, were characterized with varying degrees of inhibitory effects on microbial growth (Aera Jang, Jo, Kang, & Lee, 2008). In the case of dry-cured ham, the pentapeptide RHGYM, isolated from it, demonstrated efficacy in preventing the growth of *Listeria monocytogenes* (Castellano et al., 2016).

### 5.3. Anti-diabetic activity

The incidence of diabetes has reached the level of a pandemic in developed countries. Diabetes is a group of diseases related to an inability to react to insulin. Among the different types of diabetes, the most frequent are type 1 diabetes resulting from an inability to deliver insulin and type 2 diabetes resulting from insulin resistance (approximately 90% of people have diabetes type 2 and around 8% have type 1). One of the novel treatments consists of orally administered DPP-IV inhibitor activity. DPP-IV administration is beneficial for glucose regulation, slowing down the inactivation of hormones glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1, which enhance insulin secretion (Lacroix & Li-Chan, 2012). Numerous examples of bioactive peptides containing DPP-IV activity were identified from meat and by-products resulting in advantages for diabetes disease, although not as much as chemically synthesized molecules (Bechaux et al., 2019). In this sense, it was reported that pork meat is a potential source of bioactive peptides to control blood glucose levels as demonstrated by *in silico* analysis (Kęska et al., 2019). Supporting this study, it was suggested that several peptides identified in a dry-cured ham could be used as DPP-IV inhibitory peptides in functional foods or pharmaceutical products (Gallego, Aristoy, & Toldrá, 2014; Mora, González-Rogel, Heres, & Toldrá, 2020).

### 5.4. Antihypertensive activity

In the health field, inhibitors of angiotensin I converting enzyme (ACE) required a great deal of attention for prevention of the hypertension. The ACE does not catalyze the conversion of angiotensin I to angiotensin II provoking hypertension. ACE inhibitor peptides competitively prevent the binding of angiotensin I to ACE, inhibiting the production of angiotensin II, a strong vasoconstrictor, while medicines generally block ACE and interfere with its function. In numerous animal species, peptides with the same sequence and ACE inhibitory action have been found. For instance, in Kacang goat and wild swine muscles, it has been reported that the tetrapeptide FQPS showed an ACE inhibitory activity of  $\text{IC}_{50}$  of 27.0  $\mu\text{g}/\text{mL}$  (Mirdhayati, Hermanianto, Wijaya, Sajuthi, & Arihara, 2016). In other species, such as bovine (*biceps femoris* muscle) the hexapeptide VLAQYK displayed ACE inhibition value of  $\text{IC}_{50}$  value of 23.11  $\mu\text{g}/\text{mL}$  (Jang & Lee, 2005). Furthermore, in low-value cuts of bovine (*pectoralis profundus*), the sarcoplasmic hydrolysates below 3 kDa displayed ACE inhibitory activity (specifically attributed to peptides INDPFIDLHYM and RGDGLIEPAEKVF). The explanation is based on their hydrophobic amino acids (methionine and phenylalanine) at their termini carbon, which may influence positively the ACE-inhibition (Di Bernardini et al., 2012).

## 6. Potential applications of peptides

### 6.1. Food applications

There is growing interest in consuming suitable amounts of nutrients and bioactive compounds because of their potential to prevent non-communicable diseases. According to Chakrabarti, Guha, and Majumder (2018), bioactive peptide-based foods can be classified into several categories based on their production method. While some traditional foods such as meat, milk, legume, and fish can be valuable but not always the healthiest option, they may contain ingredients for

individual needs enriched with a wide range of bioactive peptides. Bioactive peptides can be incorporated into diets for specific purposes, such as supporting vulnerable populations, including children and the elderly, under strict regulations. Therefore, bioactive hydrolysates are a promising option for creating supplemented food tacking positive physiological impacts and health benefits. For instance, antidiabetic peptides could be added to sausages to create a product that offers health benefits by helping to prevent diabetes as they can provide a convenient way to incorporate relevant biopeptides into human diets (Bechaux et al., 2019).

On the other hand, biopeptides have the potential to enhance the quality and shelf life of meat products in terms of sensorial attributes such as taste, odour and texture. Antioxidants are widely used compounds to prevent lipid and protein oxidation. Specifically, meat proteins could undergo chemical changes like oxidation and nitrosation, reducing their nutritional value and impairing protein digestion. The growing demand from consumers for natural additives has compelled the food industry and researchers to explore alternative methods to obtain natural antioxidants (de La Pomélie, Santé-Lhoutellier, Sayd, & Gatellier, 2018). This fact highlights the importance of using natural food additives as peptides with antioxidant properties. In this regard, several researchers evaluated the effectiveness of using starter cultures to release antioxidant peptides from meat proteins and reduce oxidative damage during the production of dry-cured hams. It tested isolated and combined microorganisms including *Lactobacillus acidophilus* and *Bifidobacterium animalis*, to enhance the antioxidant activity during the ripening process of the hams. The study demonstrated the potential of starter cultures as a natural strategy to improve the quality and shelf life of meat products (Okon, Stadnik, & Dolatowski, 2017). The potential of peptides as food antioxidants have been further demonstrated in a recent trial involving minced beef. Pepsin has been utilized to hydrolyzed bovine haemoglobin obtained from a local slaughterhouse and isolate the peptide TSKYR. Since added as a preservative to meat products at a concentration of 0.5% (w/w), this peptide was found to significantly reduce meat rancidity and lipid oxidation by up to 60%. These findings emphasize the promising technological applicability of peptides in the food industry. In meat products, the main mechanisms of peptides for preventing lipid oxidation are through metal ion chelation, inhibition of lipid peroxidation and radical-scavenging properties (Sohaib et al., 2017). Therefore, peptides can be used in the food industry as natural preservatives or flavour enhancers as well as to improve the texture and nutritional value of meat products.

## 6.2. Challenges and limitations

The major limitation of biopeptides to health promotion is that their bioavailability is extremely low. The food matrix-peptide interactions and reactions of nucleophilic moieties can reduce bioavailability (Udenigwe & Fogliano, 2017). The presence of emulsifiers, metal ions, fats, biopolymers and others could interact with bioactive compounds sequestering them and avoiding their free diffusion through the extracting digestion liquid. Thus, carnosine is efficiently liberated from the food model after digesting inside the gastrointestinal tract, allowing for optimum bioavailability and absorption. As a result, carnosine absorption in the small intestine is mostly mediated by peptide transporters connected to protons PepT1 (Marcolini et al., 2015). There is a need for improvement in the current legal framework to facilitate the introduction of biopeptides into the market. Bioactive peptides derived from traditional sources of protein such as milk, whey, fish, and soybean are already widely consumed. However, it is important to conduct further studies, including *in vitro* and animal studies, to establish the health benefits of these bioactive peptides. Also, ensuring the safety of peptides is essential, which includes verifying their lack of toxicity, cytotoxicity and allergenicity.

In summary, while bioactive peptides from common protein sources have already gained popularity, their full potential should be explored.

The legal framework needs to be updated to make it easier to bring these promising products to market, and further research is needed to provide robust evidence of their health benefits.

## 7. Conclusions

This review highlights the benefits and potential applications of peptides derived from meat and its by-products. It covers both conventional and green extraction methods from *in silico* approaches for peptide identification. The bioactive hydrolysates could be utilized as food additives in new formulation products to enhance their functional and nutritional properties. Additionally, new bioactive peptides derived from meat and its by-products were reviewed for their potential applications in food and pharmaceuticals. However, it is important to validate the bioactivity of peptides as well as the bioavailability using *in vivo* assays. Furthermore, clinical studies are needed to determine the appropriate dosages, dose-response relationships, and interaction with the food matrix.

## Credit author statement

María López-Pedrouso: Conceptualization, Data curation, Writing - review & editing.

Ahmed A. Zaky: Conceptualization and Writing - review & editing.

José M. Lorenzo: Supervision and Writing - review & editing.

Mercedes Camiña: Data curation and Writing - review & editing.

Daniel Franco: Conceptualization, supervision, Writing - review & editing.

## Declaration of Competing Interest

The authors declare that there is no conflict of interest with this manuscript.

## Data availability

Data will be made available on request.

## References

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