AGRO PRODUCTIVIDAD

Bioactive peptides in red meats, byproducts and residues from the meat industry

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ABSTRACT

Objective: To provide a comprehensive overview of the generation of bioactive peptides from raw red meat, its by-products, and the waste meat cuts, as well as the bioactive health effects.

Design/methodology/approach: A literature search was conducted through the organization and systematized analysis of information for an updated literature review of bioactive peptides in red meat.

Results: Meat is a food of great nutritional value for human beings, since the digestibility of its protein stands out. In addition to the nutritional value of this protein, there are some studies in meat where bioactive peptides provide bioactivity mainly as an antihypertensive and antioxidant. Bioactive peptides are generally obtained by enzymatic hydrolysis and through microbial fermentation processes. Subsequently, they are identified by analytical techniques to perform *in vitro* and *in vivo* evaluations verifying the bioactivity of the peptides obtained.

Limitations on study/implications: The food industry must establish the most reliable methods for generating more reliable peptides to standardize their production and avoid process variability.

Findings/conclusions: The bioactive activity to be exerted by the peptides will depend on factors specific to the protein, such as the number of amino acids it contains, the hydrophilic or hydrophobic profile, and size, among others. This information is key to predict the activity the peptide can exert in the human body since many can have multiple activities, the most common being the antioxidant-antihypertensive function.

Keywords: bioactive peptides, meat, antioxidant, antihypertensive.

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INTRODUCTION

Meat is a food of great nutritional contribution for human beings, and its absence from the diet can cause diseases related to malnutrition such as metabolism disorders, thrombosis, anemia, among other clinical ailments that affect the population in general [1,

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2]. The lack of nutrients that meat consumption contributes, such as high-bioavailability proteins, essential amino acids, and vitamins can affect to a greater extent the sectors of the population that need them most, for example developing children, pregnant and lactating women, as well as the elderly, because they are not easily found in other food groups [3]. Derived from these proteins, smaller peptides are obtained which contribute essential amino acids for the correct function of the body; however, there are peptides with punctual characteristics which, in addition to the nutritional contribution, provide a beneficial activity as antihypertensive, antimicrobial, anti-inflammatory, antidiabetic, hypocholesterolemic, antioxidant, antithrombotic, and anticancer, favoring the consumer's health [4]. The production of bioactive peptides in meat happens naturally during digestion in the gastrointestinal tract, in food processing, or hydrolytically ex situ by adding microorganisms or substrates capable of generating proteolytic reactions that reduce the sizes of the protein chains and liberating smaller peptides with bioactive activity [5, 6]. Maturing meat is a necessary process to elaborate meat products, and during this stage the moisture content is reduced, generating breakdown of muscle fibers which affect the length and amount of peptide chains which is reflected in the concentration of bioactive peptides. Currently, the most common ways of conserving meat are storage and transport in refrigeration and/or freezing, and for this reason, it is essential to understand the behavior of the reactions that are generated internally at low temperatures for the generation of bioactive peptides in meat [7, 8].

Therefore, the objective of this study is to gather relevant and updated information about the generation of bioactive peptides in fresh red meats, its byproducts and waste meat cuts such as bones, blood and skin. Through this literature review, a comprehensive description is offered of the bioactive peptides in red meats and of the possible bioactive effects on health.

Peptides in meat and meat products

In particular, red meats are sources of high-quality proteins, contributing on average 20-25 grams of protein for every 100 grams of raw meat, in addition to providing valuable nutrients such as minerals and vitamins, which is why the adequate consumption of meat together with a mixed diet ensures the supply of nutrients [1, 2]. It should be highlighted that the different species destined to the consumption of meat will present different peptide profiles and this is because of factors such as race, sex, age, weight, and metabolic differences between species [9].

Similarly, any conserved and/or processed meat through salting, curing, chemical addition, smoking or any other method that has the benefit of giving added value to the natural cut of meat is considered to be a meat product; and during these processes, the concentration of the water activity is reduced, increasing proteolysis [10]. Meat products are foods that contribute a high content of proteins; however, as a result of the conservation methods and additives commonly used, their salt content is very high and their consumption has been related to health problems like high blood pressure and cancer [11].

The processing of meat products cured dry reduces the activity of water in meat to mature it, and proteolysis begins as a result of the actions of muscular endogenous peptidases such as calpains and catepsins, as well as microbial peptidases that generate free amino acids and peptides of low molecular weight [13]. The amount of bioactive peptides produced during the elaboration of a meat product will depend on the physical and chemical treatments which the meat must undergo, such as changes in temperature, time, moisture and pH, since they alter the structures and break the peptide chains, altering the concentration of peptides [14].

Dry-cured meat products as a natural source of antihypertensive and antioxidant peptides are of interest, because they could help to counteract the adverse action of sodium chloride they contain, thus helping to maintain a stable blood pressure and, therefore, a good state of health [2].

Elaborating meat products implies performing some sort of treatment (acid, alkaline, thermic, etc.) on meat, and this process affects the functional and nutritional properties because it modifies the presence of amino acids. Acid treatments destroy asparagine and glutamine, while alkaline treatments destroy cystine, serine and threonine, reducing the presence of bioactive peptides in the final product [15]. The constant variations of temperature and acidity during meat processing also affect the content of bioactive peptides, particularly if sugars have been added since the bioavailability of lysine decreases drastically when Maillard reactions are generated during cooking [9]. Structural changes during the oxidation of meat proteins and meat products can alter the rate of hydrolysis and affect the bioavailability of amino acids, and consequently, the yield of bioactive peptides will be low [12].

Methods for obtaining bioactive peptides

Because peptides are encrypted in a mother protein, the techniques to produce them should be specific and selective to promote an adequate liberation of peptides; it can be done through the action of endogenous enzymes during the processing, the gastrointestinal digestion in the consumer's tract, or the use of commercial exogenous enzymes [16]. There are also peptides that are produced by bacteria, which generate two variants of peptides, ribosomal peptides and non-ribosomal peptides, the latter obtained from enzymes and multi-enzymatic complexes [17]. In the industry, protein hydrolysis can be done with acids, alkali metals or enzymatic agents to generate bioactive peptides from meat, meat products and byproducts [18]. The use of endogenous proteins, that is, those from meat, is limited to certain products such as those cured and matured since the activity of these enzymes is limited and the yield of obtaining peptides is relatively low [19]. Therefore, the use of exogenous enzymes that have a greater activity is preferred, which are extracted from plants, microbes or animal tissues. The selection of dietary proteins from which peptides will be obtained is determined through the criteria of whether they come from a byproduct that can give added value and if the production of such peptides has a pharmacological interest [20].

Different exogenous enzymes have specificity for proteolysis, the selection of the correct enzyme will influence the sequence of amino acids from peptides that give rise to different nutritional, functional and bioactive properties in the hydrolysates [21, 22].

The most frequently used traditional techniques for the generation of peptides in the industry are hydrolysis and fermentation, from which free amino acids and short-chain peptides are obtained, and the ultra-filtration is also used when an estimated molecular weight of the peptide fractions is sought. When it comes to peptide purification, high-resolution liquid chromatography is the most used technique because a short time is needed and compared to other techniques, it can produce peptides at a large scale; this technique is supported by detectors of ultra-violet (UV) rays and fluorescence for the detection of peptides [23, 24].

The peptides are obtained from proteins through *in vivo* and *in vitro* hydrolysis or during the fermentation of foods using proteolytic cultures [20]. In addition, the hydrolysis of meat proteins results in polypeptides with a smaller number of amino acids than the original protein, and when hydrolysis continues, smaller peptides are liberated until they reach tripeptides, dipeptides, and free amino acids [25].

A low-cost technique of bioactive peptide production used is microbial fermentation using feed-grade strains where microorganisms liberate proteases to hydrolyze extracellular proteins that they use as source of nitrogen, resulting in the liberation of short-chain peptides and although their use is focused on dairy products, they also give good results in meat products such as chorizo [26, 27].

Identification of bioactive peptides

To identify the sequences of peptides, it is fundamental to analyze the protein sequences. For this purpose, a variety of techniques are used jointly to achieve better results, the most frequent combination being liquid chromatography and mass spectrometry [28].

In fermented products, the production and characterization of new peptides will depend on the type of substrate, microbial strain, proteolytic enzyme, and conditions that must be guaranteed while obtaining them, such as pH, time-temperature rate, and enzyme/substrate concentration which optimize the maximum activity of the enzyme to obtain a higher yield. However, this methodology lacks standardized protocols for the evaluation of biological activities [21, 29].

Although microbial fermentation and enzymatic hydrolysis are the most demanded techniques for the production of bioactive peptides due to their relatively simple and safe processes, the thermal processes should be supervised to avoid alterations in the structures of amino acids such as racemization, decomposition, dehydration (primarily serine and threonine), cycling of Beta eliminations, or glycation, because they can affect the functionality of peptides [30].

In addition to the techniques to obtain bioactive peptides of natural origin, it is also possible to synthesize them through chemical, enzymatic or recombinant DNA synthesis if their amino acid composition is known [31].

Various methodologies have been used to identify the residues generated by proteolysis of the muscle proteins; the use of mass spectrophotometry in tandem is the most used technique to determine the peptide sequences generated during the natural processes of protein hydrolysis; electrophoresis in polyacrylamide gel helps to separate larger proteins and to identify them through fingerprints of peptide masses with matrix-assisted desorption/ionization laser mass spectrophotometry [32].

Reversed-phase high-performance liquid chromatography is the technique that is mostly used to characterize the sequences of bioactive peptides and together with mass spectrometry it can undo the sequence by molecular weights; despite being a viable solution, the costs are high so other purification methods have been chosen such as ultrafiltration, gel chromatography, ionic exchange, and exclusion by size, among others [14]. After synthesizing the bioactive peptides, the greatest problem for the industry is the application in foods, because of the bitter taste they have, low stability in food matrices and susceptibility to gastrointestinal digestion.

New techniques to obtain and to process peptides emerge as scientific discoveries advance, which have shown to be environment-friendly, sustainable and efficient; among them, there is extraction assisted by microwaves and by ultrasound. Regretfully, the costs of specialized equipment are too high, so standard techniques which have been given good results for years are still being used [24].

Another of the methodologies implicated for the identification of residues generated in proteolysis is the in silico predictive analysis, which allows detecting the bioactive peptides of several proteins with the help of databases to track the original proteins from which short amino acid sequences were originated and which also help to predict the potential biological activity that they can have [14].

To evaluate in vivo the stability of bioactive peptides during digestion is a complicated task, since doing it represents experimenting in humans which can be invasive and uncomfortable [6, 33]. For this reason, *in vitro* digestion tests are carried out to evaluate the stability and the actual beneficial activity of the peptides, in simulated digestions tests where scenarios are set out that imitate the conditions of a digestive system, such as the enzymes and the optimal time [34]. This represents one of the greatest challenges for the food science branch, because of the inability for a greater amount of bioactive peptides to remain intact through the digestive tract. Despite this, there are promising peptides such as lactotripeptides Ile-Pro-Pro and Val-Pro-Pro which have proven to keep their native structure, and they are being tested in commercial products [35].

Meat wastes

In the meat industry, animal waste byproducts are generated while obtaining meat, such as esquilmos (parts of the animal that are not used to elaborate meat products), and secondary meat cuts abundant in fat with low commercial value, among others [36].

The blood, non-edible organs, skin, horns, hoofs, bones, fat tissues are considered useless elements for the large trade of meat cuts, which does not mean that they are not useful; the functional value of their proteins continues to be very high since there are proteins of animal origin from which protein concentrates can be obtained with multifunctional activities such antioxidant and antihypertensive [37, 38]. In rigid structures such as connective tissue, bones and cartilage from vertebrates there is a functional fibrous protein complex that makes up close to a third of all the body proteins called collagen; its structure is made up mainly by glycine molecules and other amino acids in three intertwined chains forming a triple helix, which can be split by exogenous proteases to generate bioactive peptides in foods, and the main application is in gelatin elaboration [21]. In a study carried out with collagen obtained from pork skin, it was identified that the sequence Gln-Gly-Ala-Arg has positive effects when the oxidation speed of linoleic acid and the elimination of free radicals slow down [39].

The main technique to obtain hydrolisates from this structure is enzyme proteolysis and studies have been centered in their antioxidant and antihypertensive activity because of its constant sequences of Gly-Pro-Hyp that can unleash these potential biological activities [38]. The aromatic amino acids tyrosine, tryptophan and phenylalanine have the capacity to act as eliminators of radicals. In particular, the antioxidant activity of tyrosine associated with the ability of phenolic groups to act as hydrogen donators, thus deactivating the free radicals [40]. Antioxidant peptides with leucine in their N-terminal carbon, such as Leu-Asp-Gln-Trp, Leu-Pro-His-Ser-Gly-Tyr and Leu-Leu-Gly-Pro-Gly-Le-Thr-Asn-His-Ala, can improve the capacity to capture electrons in the peptide interaction while other dipeptides mostly identified as histidine, carnosine and anserine have been highlighted because of their demonstrated antioxidant activity [2, 14].

Table 1. Antioxidant bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
DSGVT; IEAEGE; DAQEKLE	Porcine muscle *longissimus dorsi*	Saiga, Tanabe y Nishimura [44]
DLYA; SLYA; VW	Porcine muscle	Arihara y Ohata [45]
ALTA; SLTA; VT	Porcine muscle	Arihara [46]
NR	Porcine blood	Chang *et al.* [47]
QGAR	Pork meat	Li *et al.* [39]
NR	Porcine plasma	Wang *et al.* [48]
MQIFVKTLTG	Deer muscle	Kim *et al.* [49]
NR	Porcine plasma	Xu *et al.* [50]
NR	Pork meat	Liu *et al.* [51]
QYDQGV; YEDCTDCGN; AADNANELFPPN	Buffalo horn	Liu *et al.* [52]
NR	Pork liver	Di Bernardini *et al.* [53]
FGG; DM	Pork sausage	Broncano *et al.* [54]
NR	Porcine blood	Alvarez *et al.* [55]
SAGNPN	Pork cured ham (Landrace-Large White)	Escudero *et al.* [56]
QYP	Fermented ground pork sauce	Ohata *et al.* [57]
DLEE	Xuanwei cured pork ham	Xing *et al.* [58]
GKFNV; LPGGGHGDL; LPGGGT; HA	Jinhua pork ham	Zhu *et al.* [59]
SNAAC	Pork cured ham (Landrace-Large White)	Gallego *et al.* [60]
AEEEYPDL	Pork cured ham (Landrace-Large White)	Gallego, Mora y Toldrá [61]

NR: Unreported peptide sequences.

Table 2. Antihypertensive bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
MNPPK; ITTNP	Porcine Myosin Thermolysin Hydrolysate	Arihara *et al.* [62]
ITTNP	Hydrolyzed porcine myosin thermolysin hydrolysate	Arihara *et al.* [62]
RMLGQTPTK	Pork loin (*Longissimus dorsi*)	Katayama *et al.* [63]
VLAQYK	Beef meat	Jang *et al.* [64]
VLAQYK	Beef meat	Jang *et al.* [65]
SPLPPPE; EGPQGPPGPVG; PGLIGARGPPGP	Beef muscle	Bauchart *et al.* [66]
LGFPTTKTYFPHF; VVYPWT	Pork blood	Yu *et al.* [67]
VKKVLGNP	Pork meat	Katayama *et al.* [68]
GFHI; DFHING; FHG; GLSDGEWQ	Beef	Jang *et al.* [69]
KRQKYDI	Pork meat	Katayama *et al.* [70]
KRVITY	Pork meat	Muguruma *et al.* [71]
KAPVA	Pork long back muscle	Escudero *et al.* [72]
TKAVEHLDDLPGALSELSDLHAHKLRVDPV NFKLLSHSLL; LDDLPGALSELSDLHAHKLRVDPVNFKLLS HSL; KLLSHSL; LLSHSL	Beef blood	Adje *et al.* [73]
AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP; PAGNPGADGQPGAKGANGAP	Beef calcaneal tendon collagen	Banerjee y Shanthi, [74]
RPR; KAPVA; PTPVP	Pork meat	Escudero *et al.* [75]
AAATP	Pork cured ham (Landrace-Large White)	Escudero *et al.* [76]
FQPS	Goat meat	Mirdhayati *et al.* [77]
NR	Jinhua pork ham	Zuo *et al.* [78]

NR: Unreported peptide sequences.

On the other hand, thousands of liters of blood are obtained as byproduct from animal slaughter for consumption, and this liquid can represent an environmental problem if it is not managed with due care [36]. Antimicrobial benefits have been found in different studies, mainly from blood, because it is rich in proteins structured by hemoglobin [36]. In the same way, bioactive peptides have been obtained from waste material, through hydrolysis of proteins from skeletal muscle and connective tissues of some species such as pig and chicken [37].

Beneficial activity of bioactive peptides

With the scientific advances it has been discovered that peptides from the food proteins are physiologically active in a direct way, through their presence in the undisturbed food itself or after their liberation during the consumer's digestion, both *in vivo* or *in vitro* [41]. The bioactive compounds accompany the nutritional structures in foods that provide nutraceutical factors in favor of the consumer's health; they are found in low concentrations so it is necessary to have a constant intake to potentiate their effects [42]. The main intention of generating bioactive peptides is to seek the benefit to human health, although their safety should be verified, preventing them from being allergenic and/or toxic for their consumption, since in most cases they are produced with enzymes that are not found within the human digestive system; in addition, these peptide sequences do not have a record of safe use [21]. A problem that bioactive peptides face is that they must resist the digestion process, be absorbed in the intestinal epithelium, and distributed by the bloodstream to exert their physiological function in the target organ; for example, peptides with proline residues are more resistant to attacks from gastrointestinal enzymes so they can be absorbed in a nearly intact manner [43, 16]. Antioxidant and antihypertensive activities are of special interest in the study because hypertension and cell oxidation play an important role in the development of cardiovascular diseases, which is why the incorporation of bioactive peptides to the diet can prevent these diseases [9].

Table 3. Opioid bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
YPWT; YPFT	Beef blood	Brantl *et al.* [79]
LVVYPWTQRF; VVYPWTQRF	Beef blood	Piot *et al.* [80]
Hemorfina 7 LVV & Hemorfina 7 VV	Beef blood	Zhao *et al.* [81]
LRFPMQR; LVVYPATQR; LVVYPATQRFFE; VVYPATQR; DVGQTVDDPYA; DLHAYKLRVDPVNFKLLSH; FRLLGNVL; VVYPWTQRF; LEGKVLPGVDA; KHVAGAAAAGAVVGSLGGYM; YPWVAD; GVGVAPGVGVAPGIGLGPGGVIA	Lamb brain	Ianzer *et al.* [82]

Table 4. Antimicrobial bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
FLSFPTTKTYFPHFDLSHGSAQVKGHGAK	Beef blood	Fogaça *et al.* [83]
VLSAADKGNVKAAWGKVGGHAAE	Beef blood	Froidevaux *et al.* [84]
VTLASHLPSDFTPAVHASLDKFLANVSTVL	Beef blood	Daoud *et al.* [85]
VTLASHLPSDFTPAVHASLDKFLANVSTVLTSKYR; TSKYR; STVLTSKYR; QADFQKVVAGVANALAHRYH	Beef blood	Nedjar-Arroume *et al.* [86]
SHLPSDFTPA; VHASLDKFLA	Beef blood	Nedjar-Arroume *et al.* [87]
GFHI; DFHING; FHG; GLSDGEWQ	Beef meat	Jang *et al.* [69]
KYR; SKYR	Beef blood	Catiau *et al.* [88]
VNFKLLSHSLLVTLASHL	Beef blood	Hu *et al.* [89]
YSKYR	Beef blood	Przybylski *et al.* [90]

Table 5. Antithrombotic bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
NR	Pork meat	Morimatsu *et al.* [91]
NR	Pork meat	Shimizu *et al.* [92]

NR: Unreported peptide sequences.

Table 6. PEP-inhibitor bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
MPPPLPARVDFSLAGALN	Bovine brain	Ohmori *et al.* [93]

CONCLUSIONS

The bioactive activity of peptides depends on factors of the protein, such as the amount of amino acids that it has, its hydrophilic or hydrophobic profile, and size. This information is key to predict the activity that the peptide can exert in the human body, since many of these can have multiple activities, with the most common being antioxidant antihypertensive. The incorporation of meat cuts and inedible parts of animals such as skin, blood, horns and bones to the production of bioactive peptides has opened the possibility of taking the maximum advantage of the consumption of animals for human benefit, and this way to significantly reduce the environmental impact of the industry. The bioactive peptides function as auxiliary in the improvement of the state of health and can also be useful in the treatment of diseases; however, the pharmaceutical industry is facing the challenge of increasing the research on this issue of proteomic applied to medicine, since critical information is unknown to define bioactive peptides as a solution in disease treatment and recovery. Likewise, the food industry should establish methods to generate more reliable peptides to standardize their production and to avoid the existence of variability in the processes.

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