Meat and Muscle Biology™

Bioactive Peptides in Meat and Meat Products

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Abstract: A number of bioactive peptides with health benefits have been reported to be generated in meat through the proteolysis phenomena taking place during postmortem aging or further processing. Bioactive peptides consist of short sequences, less than 20 amino acid residues, that are inactive while in the parent protein. These sequences may be activated when they are released from the parent protein by peptidases. Such peptides are released through the hydrolysis of major muscle proteins by muscle endopeptidases, like calpains and cathepsins, and resulting protein fragments and polypeptides are further hydrolyzed by muscle exopeptidases, mainly, tri- and di-peptidylpeptidases, aminopeptidases, and carboxypeptidases. As a result, a variety of peptides with different sequences and lengths and large amounts of free amino acids are generated. The highest amounts of bioactive peptides, and their major health benefits, have been reported in dry-cured ham as a result of the intense proteolysis experienced during the long processing time under mild conditions that favor the action of muscle peptidases. The generated peptides must be bioaccesible and bioavailable to exert their physiological benefit. This means that they must be resistant to gastrointestinal digestion and be absorbed through the intestinal barrier. Some bioactive peptides may be generated through hydrolysis during gastrointestinal digestion. This review is focused on the generation of bioactive peptides in meat and processed meats, on the most relevant bioactivities exerted by such peptides (such as inhibition of angiotensin I–converting enzyme and dipeptidyl peptidase IV as well as antioxidant and anti-inflammatory activity), and on their reported benefits to consumers' health.

Key words: bioactive peptides, hydrolysis, peptidase, proteomics, peptidomics, health
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Introduction

Meat constitutes a very important food in our diet because it contributes to the intake of high-quality proteins containing all necessary essential amino acids as well as important micronutrients such as iron, zinc, selenium, magnesium, as well as vitamins B12 and B6, niacin, choline, riboflavin, among others. Further to the well known nutritional content of meat, a number of bioactive peptides with health benefits have been reported to be generated in meat through the hydrolysis of muscle proteins by endogenous peptidases. Such bioactive peptides consist of short sequences (2–20 amino acid residues) that remain inactive while encrypted within the parent protein but that could be activated when released by peptidases during either postmortem aging or further meat processing, or even during the gastrointestinal digestion (Toldrá et al., 2018). Therefore, the generation of bioactive peptides in meat or processed meat is the result of a cascade of enzymatic reactions, initiated by the action of muscle endopeptidases, majorly calpains and cathepsins, that contribute to break muscle proteins into major protein fragments and polypeptides. Such fragments are then further hydrolyzed by muscle exopeptidases like tripeptidylpeptidases and dipeptidylpeptidases into smaller peptides, and by aminopeptidases and carboxypeptidases into free amino acids (Toldrá et al., 2020a). An example of how the different muscle peptidases may act on a fragment of myofibrillar protein is shown in Figure 1.

Bioactive peptides may exert different physiological regulatory activities that promote consumers' health, but the effects depend on its molecular size,

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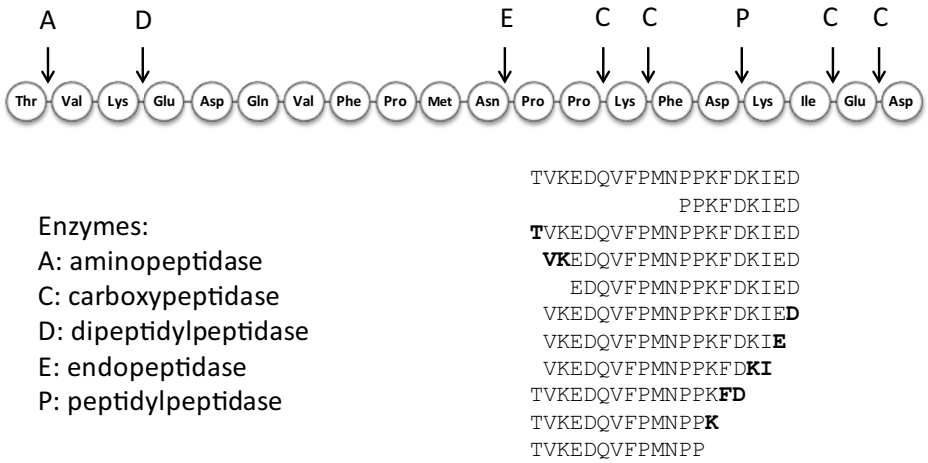

Figure 1. Scheme of food protein hydrolysis and enzymes involved. The amino acid sequence is a fragment belonging to the myosin heavy chain. This figure was adapted from Mora et al. (2013) with permission from Elsevier.

spatial structure, amino acid composition, and hydrophilic and hydrophobic properties. They must also be resistant to gastrointestinal digestion and be absorbed intact through the intestinal barrier, showing good bioaccesibility and bioavailability to exert their physiological benefit. Most usual bioactivities reported in meat are related to peptides with cardioprotective action like the inhibition of angiotensin I–converting enzyme (ACE) and antioxidant activity (Xing et al., 2019), but other activities like anti-inflammatory and dipeptidyl peptidase IV (DPP IV) inhibition has also been reported in all types of meats (Madhu et al., 2022; Ashaolu et al., 2023).

This review is focused on the generation of bioactive peptides by endogenous peptidases in meat and meat products, their major types of bioactivity, and the reported benefits for consumer health.

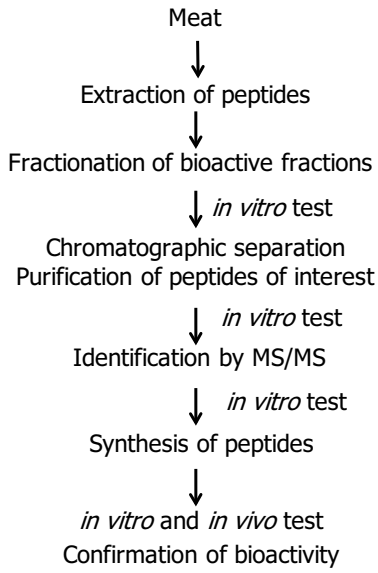

Figure 2. Scheme of the traditional empirical procedure for the identification and confirmation of bioactive peptides from food matrices. MS/MS, mass spectrometry in tandem. Reproduced with permission from Mora et al. (2018).

Bioactivity Prediction of Released Peptides

Bioactive peptides are usually identified in meat and processed meat products through empirical approaches as schematized in Figure 2. This process involves the extraction of bioactive peptides and their separation through chromatographic techniques by collecting fractions and screening bioactivity in order to select the most active fractions for further purification of peptides. The purified peptides are usually identified using mass spectrometry in tandem, and the most active sequences are selected for synthesizing peptides to be used in confirmatory *in vitro* and *in vivo* assays (Sánchez-Rivera et al., 2014; Mora et al., 2018). This procedure is tedious and costly and therefore it may be complemented with predictive strategies based on *in silico* analysis using bioinformatics tools and peptide databases as shown in Figure 3 (Lafarga et al., 2014; Mora et al., 2018). BIOPEP-UWM is a database used for *in silico* approach and bioactivity prediction (Minkiewicz et al., 2019). The quantitative structure–activity relationships (QSAR) model and molecular docking simulations are useful for the characterization of structural and physico-chemical properties (Carrasco-Castilla et al., 2012; Agyei et al., 2016). In this way, the combined use of empirical and *in silico* approaches facilitates the location of peptides and the determination of their potential bioactivities in

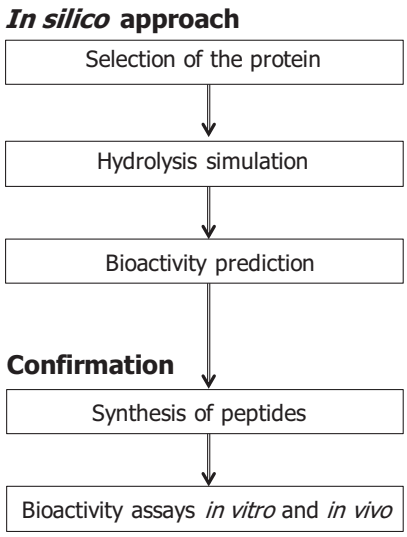

Figure 3. Main steps of *in silico* approaches and open access databases for the selection of the protein, hydrolysis simulation, and bioactivity prediction. Adapted with permission from Mora et al. (2018).

complex matrices like meat and processed meats (Toldrá and Mora, 2022).

The bioavailability of bioactive peptides is assessed in order to ensure that the specific bioactive peptide keeps its bioactivity during gastrointestinal digestion, crossing through the intestinal membrane and flowing within the bloodstream until reaching the target organ (Segura-Campos et al., 2011). Simulated gastrointestinal digestion is usually performed under standard protocols with specific enzyme conditions. The ability to transport peptides through the intestinal epithelium can be assayed using a Caco2 cell monolayer (Gallego et al, 2016; Wang and Li, 2017). Finally, *in vivo* assays are necessary to confirm that peptides are not degraded by blood plasma peptidases (Bohn et al., 2018).

Bioactive Peptides in Meat

Postmortem aging of meat is well known to improve tenderness, but it can also contribute to generate peptide fractions, some of them with bioactivity and therefore with potential positive effects on health (Fu et al., 2017). Once meat is ingested, bioactive peptides must be resistant to the digestive enzymes and environmental conditions during the gastrointestinal digestion, and these peptides must remain intact when crossing the intestinal barrier and when reaching the blood stream in order to be able to exert their physiological action (see Figure 4) and its health benefit (Gallego et al., 2016).

The generation of bioactive peptides under conditions of industrial aging of meat is not abundant although certain bioactivity has been reported.

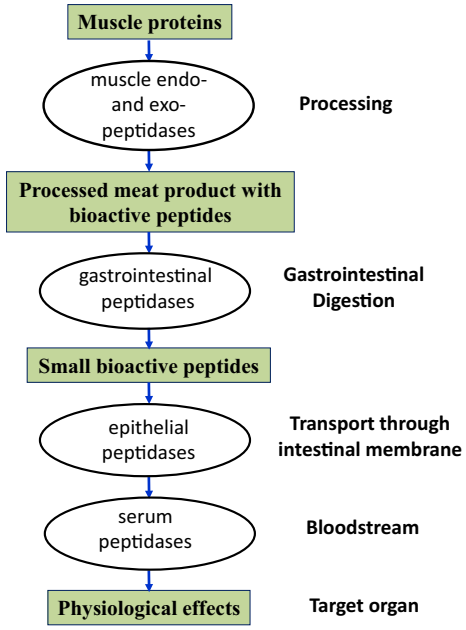

Figure 4. Scheme of the generation of bioactive peptides from meat proteins and routes followed for physiological effects.

Proteolysis mostly breaks proteins into polypeptides and relatively large peptides, also generating some peptides smaller than 3 kDa, the value of which increases with extended postmortem time (Fu et al., 2017). The contributions of such peptides to 2,2-diphenyl–1-picrylhydrazyl (DPPH) antioxidant activity as well as to ACE inhibitory activity have been reported in beef (Fu et al., 2017), pork (Escudero et al., 2012), poultry (Martini et al., 2019), rabbit (Chen et al., 2022), and chicken (Sangsawad et al., 2017). Several peptides with ACE inhibitory activity reported for fresh meat of different animal species are shown in Table 1. Cooking of meat was reported to slightly increase the bioactivity, whereas the subsequent simulated gastrointestinal digestion resulted in a substantial increase of bioactivity. Myosin, actin, titin, collagen, and elastin were reported to be the main proteins of origin for the identified bioactive peptides (Mora et al., 2017; Wang et al., 2020a). Such positive effect was attributed to changes in the conformation of proteins due to heat denaturation when cooked at 70°C that allowed for better access of the digestive peptidases to the cleavage sites of proteins (Bax et al., 2012). It must be taken into account that 1743 peptides were identified from 71 meat proteins after cooking and *in vitro* digestion (Sayd et al., 2016).

An interesting study compared the peptides generated from beef, pork, chicken, and turkey meat after their gastrointestinal digestion. More than 200 peptides were released in all 4 types of meat although only 62

Table 1. Examples of bioactive peptides generated in meat with indication of respective proteins of origin and bioactivity

Peptide sequence	Protein of origin	Meat	Type of bioactivity	Bioactivity[a] IC_{50} (μM)	Reference
KAPVA	Titin	Pork	ACE inhibitory	46.56	Escudero et al., 2010
PTPVP	"	"	"	256.41	"
RPR	Nebulin	"	"	382	Escudero et al., 2012
KRQKYDI	Troponin	"	"	26.2	Katayama et al., 2008
IPM	—	Beef	DPP IV inhibitory	70	Martini et al., 2019
IPI	—	"	"	3.5	"
AVF	Actin	"	"	406	"
LKYPI	"	"	"	27	"
LPF	—	"	"	40	"
LGI	—	"	"	50	"
WI	—	"	"	89	"
WGAP	—	Rabbit	ACE inhibitory	140.70	Chen et al., 2021
EACF	—	"	"	41.06	Chen et al., 2022
CDF	—	"	"	192.17	"
ELFIT	Myosin	Chicken	"	6.35	Sangsawad et al., 2017
KPLL	Heavy			meromyosin	"
"	11.98	"			
FHG	—	Game	"	133.8	Takeda et al., 2020
GFHI	—	"	"	310.8	"

[a]IC_{50} value is the peptide concentration that inhibits 50% of activity.

peptides matched with sequences associated to a proven biological activity such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) antioxidant activity, ACE inhibitory activity, and DPP IV inhibitory activity. Near one third of them were in common for all types of meats (Martini et al., 2019).

Recently, it has been reported that hydrolyzates of meat proteins contain cryptides that are short peptide sequences encrypted within longer peptides that need further processing, like during gastrointestinal digestion, to release their bioactivity (Gathercole et al., 2023).

Bioactive Peptides in Processed Meats

The number and amount of bioactive peptides has been reported to be very large in meat products that have been fermented or exposed to extended drying periods. In this way, bioactive peptides can be generated from muscle proteins due to the action of endogenous endo- and exopeptidases during dry-curing or combined with microbial peptidases during fermentation/ripening. Furthermore, additional peptides may be either generated or degraded by the action of enzymes of the gastrointestinal tract (Toldrá et al., 2020b).

The activity of peptidases, and therefore the extent of proteolysis, may be affected not only by many variables such as the type of ingredients and processing conditions used but also by the type of enzymes and microorganisms used in the case of fermented meats (Toldrá et al., 1993; Zhou et al., 2019). Proteolysis is quite intense in dry-cured ham due to the large length of time of processing, usually more than 9 months. Muscle endo- and exopeptidases have enough time to hydrolyze muscle proteins, releasing numerous peptides that are progressively reduced in size as the process advances. Final products of proteolysis are tri- and dipeptides and free amino acids that accumulate in large amounts by the final stages of processing (Mora et al., 2013). Therefore, a strong proteolysis is reported in the last stages of Jinhua ham due to the higher temperatures used, and this causes a large generation of dipeptides (i.e., VE, PL, AH, and AR) and tripeptides (LPK, SGL, AAP, SGV, and LHA) with 23.59% and 48.28%, respectively, of total relative peak areas (Zhu et al., 2017). The simulated gastrointestinal digestion of Italian Parma ham was reported to generate 21 dipeptides and 12 tripeptides (Paolella et al., 2015). A good number of dipeptides (i.e., TS, TL, FD, VK, AT, and QT) and tripeptides (i.e., SRE, TVQ, NAS, KIE, and GKM) were reported in Spanish dry-cured ham (Gallego et al., 2019).

Major Bioactivities of Released Peptides

Antioxidant activity

Peptides with antioxidant activity can reduce lipid and protein oxidation in meat products like dry-cured ham and dry-fermented sausages and therefore contribute to a better final quality. Typical assays used for the determination of antioxidant activity are radical-scavenging activity (DPPH), ABTS radical-scavenging activity, ferric-reducing antioxidant power, oxygen radical absorbance capacity (ORAC), hydroxyl radical-scavenging activity (OH^-), and lipid peroxidation inhibition activity in linoleic acid emulsion.

Numerous antioxidant peptides have been reported in meat products, especially dry-cured ham, as shown in Table 2. The sequences contain between 4 and 16

Table 2. Peptides with antioxidant activity identified in different types of dry-cured ham with indication of respective proteins of origin and bioactivity

Peptide sequence	Protein of origin	Dry-cured ham	Values of bioactivity[a]	Reference
DLEE	—	Chinese Xuanwei	DPPH: 74.4% at 0.5 mg/mL	Xing et al., 2016
FLKMN	Myosin light chain	Chinese Jinhua	DPPH: 65% at 1 mg/mL, OH^-: 60% at 1 mg/mL	Zhu et al., 2016
GKFNV	—	Chinese Jinhua	DPPH: 92.7% at 1 mg/mL	Zhu et al., 2013, 2016
GLAGA	Collagen VII	Spanish	RP: 0.5 AU at 1 mg/mL	Escudero et al., 2013a
LPGGGHGDL	—	Chinese Jinhua	OH^-: 85% at 1 mg/mL	Zhu et al., 2016
LPGGGT	—	Chinese Jinhua	DPPH: 65% at 1 mg/mL, OH^-: 60% at 1 mg/mL	Zhu et al., 2016
SNAAC	Myosin heavy chain	Spanish	DPPH: 95.7% at 3 mg/mL, RP: 1.7 AU at 1 mg/mL	Mora et al., 2014
SAGNPN	Integrin α-3	Spanish	DPPH: 50% at 1.5 mg/mL	Escudero et al., 2013a
AEEEYPDL	Creatine kinase	Spanish	ORAC: 960.04 nmol TE/mg, ABTS: 1474.08 nmol TEAC/mg	Gallego et al., 2018a
MWTD	—	Chinese mutton ham	ABTS: 0.4 mg/mL	Wang et al., 2020b
APYMM	—	Chinese mutton ham	ABTS: 0.12 mg/mL	Wang et al., 2020b
FWIEE	—	Chinese mutton ham	ABTS: 0.23 mg/mL	Wang et al., 2020b

[a]Antioxidant activity measured by DPPH radical-scavenging assay (DPPH), ferric-reducing power (RP), hydroxyl radical scavenging (OH^-), oxygen radical absorbance capacity (ORAC), and ABTS radical-scavenging activity (ABTS).

amino acids, and the molecular weights range from 0.4 to 2 kDa (Liu et al., 2016; Toldrá et al., 2020b), most of them generated from myosin (Wang et al., 2021; Li et al., 2022).

Peptides AEEEYPDL (Gallego et al., 2018a) and SNAAC (Mora et al., 2014; Gallego et al., 2018b) were reported to have a high antioxidant activity in Spanish ham although they were found to be degraded, and their activity almost lost, during simulated gastrointestinal digestion. The antioxidant activity of crude peptides (< 3 KDa) extracted from Xuanwei, Jinhua, and mutton hams was reported to be high (Wang et al., 2021). In fact, powerful antioxidant peptides such as FLKMN, LPGGGHGDL, LPGGGT, and LEER (Zhu et al., 2016) were reported in Jinhua ham; DLEE (Xing et al., 2016) and GKFNV (Zhu et al., 2013) in Xuanwei ham; and MWTD, APYMM, and FWIIE in mutton ham (Wang et al., 2020b). When comparing the antioxidant activity among different Chinese dry-cured hams, it was reported that peptides from Xuanwei hams had higher DPPH radical scavenging, ferric-reducing antioxidant power, and ORAC activity than Jinhua and Rugao hams. Spanish Teruel, Italian Parma, and Belgian dry-cured hams were also compared for their antioxidant profile of peptides, and all hams had 50% to 65% of DPPH radical-scavenging activity and absorbances ranging from 1.21 to 1.28 units for the ferric-reducing antioxidant activity (Mora et al., 2016).

Angiotensin I–converting enzyme (ACE) inhibitory activity

Angiotensin I-converting enzyme (ACE) is a key enzyme in the renin-angiotensin system because it converts angiotensin I into angiotensin II, which is a potent vasoconstrictor, and it is also able to degrade bradykinin in the kinin–kallikrein system. Therefore, ACE inhibitors are closely related to antihypertensive activity. ACE is a chloride-activated zinc metallopeptidase and is able to release dipeptides from the C-terminal of peptides.

ACE inhibitors are characterized for having aromatic, positively charged, and basic amino acids in the last 3 positions of the C-terminal (Gu et al., 2011; Fernández et al., 2016). ACE is inclined to bind with peptide with penultimate Pro residues (Xing et al., 2021). Numerous peptides with ACE inhibitory activity have been reported in dry-cured ham as shown in Table 3. Myosin, followed by titin, are the major proteins of origin for most of the ACE inhibitory peptides (Xing et al., 2021).

Peptides with ACE inhibitory activity from Jinhua ham were stable against processing conditions like heating up to 100°C, salt content up to 10%, pH in the range 5 to 9, and simulated gastrointestinal digestion (Zuo et al., 2017). In fact, higher antioxidant activity and ACE inhibitory activity was reported in Xuanwei hams after simulated its gastrointestinal digestion (Wang et al., 2018). Further, some peptides have been found to be multifunctional because they can exert several activities like peptide AAATP that was reported to exert ACE and DPP IV inhibitory activity (Escudero et al., 2013b). Bioactive peptides from fermented sausages should be much less intense compared to

Table 3. Peptides with ACE inhibitory activity identified in different types of dry-cured ham with indication of respective proteins of origin and bioactivity

Peptide sequence	Protein of origin	Dry-cured ham	Values of bioactivity (IC_{50})[a]	Reference
AAPLAP	Myosin XV	Spanish Teruel	14.38 μM	Escudero et al., 2014
AMNPP	Myosin 3	Spanish Teruel	304.5 μM	Escudero et al., 2014
ASGPINFT	Myosin regulatory light chain 2	Spanish	975 μM	Escudero et al., 2013a
DVITGA	Myosin light chain	Spanish	900 μM	Escudero et al., 2013a
GGVPGG	Elastin	Spanish	79.90% at 1 mM	Gallego et al., 2019
GVVPL	—	Italian Parma	956 μM	Dellafiora et al., 2015
IAGRP	Titin	Spanish Teruel	25.94 μM	Escudero et al., 2014
IKLPP	Myosin IXb	Spanish Teruel	193.9 μM	Escudero et al., 2014
KPGRP	Titin	Spanish Teruel	67.08 μM	Escudero et al., 2014
KVLPG	Phosphoglycerate kinase 1	Spanish Teruel	265.44 μM	Escudero et al., 2014
LGL	—	Italian Parma	145 μM	Dellafiora et al., 2015
PAPPK	Myosin light chain 1/3	Spanish Teruel	199.58 μM	Escudero et al., 2014
SFVTT	—	Italian Parma	395 μM	Dellafiora et al., 2015
AAATP	Allantoicase	Spanish	100 μM, SBP: −25.6 mmHg	Escudero et al., 2013b
TGLKP	Aspartate aminotransferase	Spanish Teruel	51.57 μM	Escudero et al., 2014
KAAAATP	PR domain zinc finger protein 2	Spanish Teruel	25.64 μM	Escudero et al., 2014
KPVAAP	Myosin XV	Spanish Teruel	12.37 μM	Escudero et al., 2014
PSNPP	Titin	Spanish Teruel	192.77 μM	Escudero et al., 2014
KAAAAP	Myosin light chain 3	Spanish Teruel	19.79 μM	Escudero et al., 2014
AA	—	Spainsh	110.82 μM, SBP: —	Heres et al., 2021

[a]IC_{50} value is the peptide concentration that inhibits 50% of activity.

SBP = systolic blood pressure.

dry-cured ham due to the shorter processing time. ACE inhibitory peptides are also generated in fermented sausages where the intensity of proteolysis depends on the type of starter cultures and processing conditions used and time of ripening. Peptide VALSLSRP was identified in sausages fermented with *Lactobacillus plantarum* and *Staphylococcus symulans* and showed high ACE inhibitory activity (Huang et al., 2022).

Several peptides identified in Spanish dry-cured ham, like AA, AW, and AAATP, have shown good antihypertensive activity *in vivo* when assayed with spontaneously hypertensive rats. A substantial decrease in the systolic blood pressure was reported after 8 h of its ingesta (Escudero et al., 2013b; Heres et al., 2021, 2022). There are few clinical assays reported with human volunteers to check the effects of dry-cured ham consumption on cardiovascular health. The usual hypothesis is that blood pressure should be increased due to its high salt content. A prospective cohort study with 13,900 Spanish middle-aged adult university graduates consuming 50 g/d of dry-cured ham between 1 to 5 d a week revealed that consumption of dry-cured ham at highest levels (>5 times per week) was not associated with a significantly higher risk of hypertension in comparison to low consumption (<1 time per week) (Rico-Campà et al., 2020). Furthermore, a preliminary clinical assay with 40 healthy subjects showed a non-statistical trend toward a reduction in blood pressure suggesting the need for further assays with a higher number of volunteers (Montoro-García et al., 2017). More recently, a second clinical assay was performed with 54 healthy subjects having pre-hypertension and consuming 80 g/d of dry-cured ham. The placebo was cooked ham exempt of bioactive peptides. The assay confirmed systolic and diastolic pressures experienced a significant decrease up to 2.4 mmHg in the 24 h after ingestion. Furthermore, total cholesterol levels were reported to be significantly decreased (Montoro-García et al., 2022).

Anti-inflammatory activity

Inflammation is generally induced in the immune system and affects a broad range of cells, tissues, and organs. It is involved in chronic inflammatory conditions like hypertension, diabetes, and other diseases. The assay of anti-inflammatory activity of peptides is complex mainly because there is a high diversity and complexity of the inflammatory responses (Guha & Majumder, 2018). Bioactive peptides with anti-inflammatory activity may contribute to alleviate the inflammation condition in organs (Xing et al., 2021). Chemokines and cytokines are produced and spread to organs and tissues as a consequence of acute inflammation in macrophages and monocytes. Lipopolysaccharide plays an action similar to the endotoxin, promoting inflammatory mediators like tumor necrosis factor alpha (TNF-α), interleukin (IL)-8, IL-6, and IL-1β (Xing et al., 2021).

Peptides isolated from Xuanwei dry-cured ham were assayed in a dextran sodium sulfate-induced C57BL/6 mice trial, which observed suppression of cytokines TNF-α, IL-6, and monocyte chemoattractant protein-1 (MCP-1) in the colon and sensitive amelioration of other inflammatory bowel disease symptoms such as colon shortening, tissue damage, and colonic tissue inflammation (Xing et al., 2023). Xuanwei dry-cured ham peptides were also assayed on lipopolysaccharide-induced macrophage cell model (RAW264.7 cells) and revealed a noticeable suppresing effect on nitric oxide, IL-6, and TNF-α (Xing et al., 2023).

Anti-inflammatory activity of peptides isolated from Spanish dry-cured ham were assayed through the inhibition of platelet-activating factor-acetylhydrolase (PAF-AH), autotaxin (ATX), and lipoxygenase (LOX). PAF-AH activity was inhibited up to 26.06% by 19 peptides (with FNMPLTIRITPGSKA being the most active peptide), ATX was inhibited up to 57.49% by 13 peptides (with the strongest inhibition by PSNPP), and LOX was inhibited up to 23.33% by 5 peptides (with HCNKKYRSEM having the strongest inhibitory activity) (Gallego et al., 2019).

Dipeptidylpeptidase IV (DPP IV) inhibitory activity

The inhibition of DPP IV is involved in the metabolic pathways related to glucose metabolism due to the inactivation of glucose insulinotropic peptide (GIP) or glucagon-like peptide-1 (GLP-1) hormones. The inhibition of DPP IV prevents their degradation and helps to keep an adequate amount of glucose in plasma (Keska and Stadnik, 2021), because DPP IV are inhibitors related to treatments against diabetes mellitus type 2. Several peptides from Spanish dry-cured ham, such as AAAAG, AAATP, AA, KA, and GP, were reported to have inhibitory activity against DPP IV with IC_{50} values ranging from 6.3 to 9.7 mM (Gallego, Aristoy and Toldrá, 2014). Peptide SFVTT from Italian Parma ham was also reported to inhibit DPP IV with an IC_{50} value of 0.39 mM (Dellafiora et al., 2015). Other authors reported several peptides as DPP IV inhibitors after simulated gastrointestinal digestion of dry-cured loin (Keska and Stadnik, 2022).

Conclusions

A variety of peptides with different sequences and lengths are generated in meat and processed meat products as a consequence of proteolysis by endogenus peptidases. Some of the released peptides are bioactive because they exert activities like inhibition of ACE and DPP IV as well as antioxidant and anti-inflammatory activity. Although bioactivities have been checked with *in vitro* assays and health benefits demonstrated through *in vivo* assays with animals, further clinical assays with humans are neccessary to demonstrate the health benefits for consumers.

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