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Abstract

Proposals for sustainable use of meat industry waste and by-products have seen a remarkable growth in recent decade. This paper aims to shed light on the often-overlooked realm of meat by-products, positioning them as an invaluable source of bioactive peptides and functional ingredients. It emphasized on the first part the main strategies for valorization of meat industry by-products into diverse bioactive peptides, and then it introduces in the second part the diverse and current methods of identification and characterization of bioactive peptides and protein hydrolysates. While the promise of these macromolecules is immense, the paper focuses and takes an in-depth look in the third part at the regulatory and safety barriers hindering their efficient valorization. By addressing regulatory and safety concerns, this review aims to pave the way for a more sustainable and responsible utilization of meat by-products, ensuring not only the economic viability of the meat sector, but also fostering a holistic and safe approach towards enhanced food and animal production sustainability.

Keywords: Meat industry by-products; Peptide bioactivities; Regulatory barriers to valorization; Safety barriers to valorization; Meat industry sustainability.

Introduction

In recent decades, the surge in proposals for sustainable utilization of meat industry waste and by-products has marked a significant advancement in the field [1]. In fact, the meat industry, while producing 345 million metric tons meat, produces/generates significant quantities of by-products (approximately 155 million metric tons) including as blood, skin, bones, trimmings, organs, viscera, feet, hoofs, horns, and skulls, among other materials, during the slaughtering and meat processing processes [1,2]. Therefore, it is essential to handle and dispose these materials in an environmentally friendly manner. The by-products are potential sources for several molecules, among which peptides and proteins hydrolysates that can be used in several purposes. However, there are several technical steps and challenges in utilizing meat by-products for the recovery of bioactive peptides and functional ingredients [3,4]. This can be for instance illustrated by the bibliometric map analysis highlighting the diversity of themes addressed around the recovery of bioactive peptides and/or protein hydrolysates from meat industry by-products (**Fig. 1**). In fact, the biological activities of peptides derived from animal by-products are influenced by factors like the source of the proteins (animal, organ, status of the biological samples that can be fluid (blood) or tissue…), peptide structure, amino acid sequence, molecular weight, processing conditions employed, … etc [4,5]. Moreover, achieving commercial success is currently limited due to challenges of extraction, separation (fractionation) and purification conditions, low bioavailability, insufficient clinical trials determining safety and efficacy, and regulatory restrictions [6]. The conventional processes (fermentation, ageing, homogenization etc.) employed to generate peptides results with lower efficacy. On the other hand, fermentation and enzymatic methods are confronted with the challenges in commercialization, as the processes of fermentation and enzymatic hydrolysis are unpredictable due to variable efficiency of enzymes on substrates, processing time, cost, yield, etc." [7]. Emerging technologies for conversion of protein-rich by-products into peptides require expensive equipment, time-consuming procedures, knowledge of data analysis etc. Hence, *in vitro* and *in vivo* studies, along with studies on bioavailability, toxicity, allergenicity, and synergistic effects of peptides are required, before claiming nutritional, functional, and therapeutic effects [8].

While the potential of these macromolecules is immense, however, navigating into their valorization is a complex task due to regulatory barriers. In this context, this paper places a spotlight

on the regulatory and safety barriers that impede their efficient valorization. In fact, these barriers, rooted in diverse regional standards and safety protocols, pose challenges to the efficient utilization of these valuable by-products. From stringent approval processes to varying interpretations of safety guidelines, the regulatory landscape often impedes the streamlined incorporation of meat-derived peptides and protein hydrolysates into diverse industries. Understanding and addressing these regulatory hurdles is crucial for unlocking the full potential of these by-products, promoting sustainability, and harnessing the economic and nutritional benefits they offer to diverse sectors. Ultimately, this overview aims to pave the way for a more sustainable and responsible approach to the utilization of meat by-products, ensuring economic viability and fostering holistic and safe practices in food and animal production sustainability.

Main strategies for valorization of meat by-products

Generation and extraction methods of bioactive peptides

The current methods applied to generate protein hydrolysates comprise acid-base hydrolysis, microbial fermentation, and enzymatic treatments. Among these, the acid-base hydrolysis methods are normally employed in industrial production. However, the corrosiveness of acidic and alkaline solutions poses a risk of damaging pipelines or reaction tanks, thereby imposing significant limitations on their industrial application [9]. In fact, microbial fermentation imposes stringent requirements on start culture, and the culture process for bacteria is both lengthy and intricate. In comparison with the traditional acid-base hydrolysis method, enzymatic hydrolysis is commonly used with several advantages, including mild reaction conditions, uniform protein hydrolysates, and the ability to minimize secondary degradation of peptides during enzymatic hydrolysis [10,11].

The main preparation processes of bioactive peptides are illustrated in **Fig. 2**, which include raw material pretreatment, enzymatic hydrolysis, separation and purification, bioactivity evaluation, peptide identification and activity verification. Firstly, raw material pretreatment involves procedures, such as bone grinding, freeze-drying of viscera, and homogenization. Additional pretreatment methods include blood centrifugation and decolorization. These processes contribute to the degradation of structure of the samples, hence facilitating the entry of enzymes and thereby initiating the protein hydrolysis. Enzymatic hydrolysis typically occurs in both batch reactors and continuous reactors. Upon reaching the desired degree of hydrolysis, heat treatment is normally

88 employed to inactivate the enzyme, thereby terminating the enzymatic hydrolysis reaction [12].
89 The commercial food-grade enzymes commonly used to extract bioactive peptides from meat by-
90 products include papain, pepsin, alcalase, neutrase, flavorzyme, trypsin, etc [5].

91 The resultant protein hydrolysate is subsequently subjected to centrifugation for the removal of
92 lipids and impurities, recovering the supernatant. The degree of hydrolysis and bioactivity of the
93 obtained samples are further assessed. Subsequently, the most active components are further
94 separated and purified using chromatography, membrane filtration, electrodialysis, etc [12]. For
95 instance, reversed-phase high-performance liquid chromatography (RP-HPLC) involves the
96 purification of peptides based on hydrophobicity. Membrane filtration (e.g. ultrafiltration) is
97 commonly used for separating peptides on the basis of molecular weight. Electrodialysis can be
98 employed for rapid separation of peptides according to positive and negative charges. In addition,
99 to optimize the screening process, *in silico* digestion using computer-assisted strategies has been
100 used to identify bioactive peptides from meat by-products, which among its steps involves virtual
101 screening and prediction [13]. To date, the bioactivities of meat by-products have been
102 comprehensively investigated, resulting in the identification of several novel peptide sequences
103 with antioxidant, DPP-IV inhibitory, antihypertensive, antimicrobial, anti-inflammatory activities,
104 tyrosinase inhibitory, calcium-chelating and anti-aging activities (**Table 1**).

105 *Overview of the current methods for the identification and characterization of bioactive peptides*
106 *and protein hydrolysates*

107 The characterization and identification of bioactive peptides and protein hydrolysates mainly
108 include two aspects. First, the characterization of the secondary spatial structure of short bioactive
109 peptides using Fourier transform infrared spectroscopy (FTIR), circular dichroism, Raman
110 spectroscopy, X ray diffraction and nuclear magnetic resonance (NMR). Second, the identification
111 of amino acid sequence of peptides normally employs mass spectrometry. Aubry *et al.* identified
112 the secondary structure of peptide samples through a scanning electron microscope and infrared
113 absorption spectrum [14]. Hu *et al.* measured the conformational changes in the secondary structure
114 of peptides-calcium chelate by circular dichroism, FTIR, and X-ray diffraction [15]. Fourier
115 transform infrared spectroscopy was employed to investigate the relative content of secondary
116 structures and nuclear magnetic resonance spectroscopy can be further used for both qualitative
117 and quantitative analysis of the compositional structure [16].

Mass spectrometry, in the frame of proteomics and peptidomes, is the most common method for the identification of peptide sequences [17,18]. Currently, a diverse array of mass spectrometry techniques exists, encompassing various ion sources, protein cleavage modes, and mass analyzers tailored for the structural analysis of bioactive peptides. The ion sources comprise Electron Ionization (EI), Chemical Ionization (CI), Electro-spray Ionization (ESI), Atmospheric Pressure Chemical Ionization (APCI), Laser Diode (LD), and Matrix-Assisted Laser Desorption Ionization (MALDI) [19].

The main mass spectroscopic decomposition methods of protein samples include collision-induced dissociation (CID), high-energy collision dissociation (HCD), electron capture dissociation (ECD), electron transfer dissociation (ETD), electron-activated dissociation (EAD), ultraviolet photodissociation (UVPD). There are numerous mass analyzers, encompassing single-focusing mass spectrometry, double-focusing mass spectrometry, time-of-flight, ion trap, triple quadrupole, Fourier transform ion cyclotron resonance analyzer. The characteristics of fragments and mass results acquired from the mass spectrometer are juxtaposed with the theoretical peptide spectra derived from an *in silico* digested protein database. This process facilitates the identification of the target peptide sequence [20].

Diversity of peptide bioactivities in meat by-products

Antioxidant activity

The peptides from meat by-products, such as blood, bone and viscera, have been reported to exhibit potent antioxidant capacity. Przybylski *et al.* isolated the bioactive peptide (TSKYR) from bovine hemoglobin through hydrolysis with porcine pepsin [21]. The antioxidant activity of the bioactive peptide generated by porcine pepsin was assessed, revealing a TBARS value of 1.75 MDA/kg meat when the supplementation level of the bioactive peptide was 0.5%. Notably, it exhibited comparable antioxidant effects to the widely utilized antioxidant butylated hydroxytoluene (BHT). Liu *et al.* hydrolyzed sheep abomasum using papain, resulting in the isolation of the peptide LEDGLK IDDVLK, with IC_{50} value of 0.58 mg/mL for DPPH radical scavenging activity [22]. Zhan *et al.* employed porcine plasma as the raw material, subjecting it to simulated *in vivo* digestion using pepsin and trypsin [23]. The obtained bioactive peptide YDQLPEPRKPIE exhibited notable antioxidant properties, with HRAS value of 66.89%, ABTS

RAS value of 89.37%, and DPPH value of 38.32%. The observed antioxidant effect was superior to that of glutathione. The antioxidant activity may be related to the C-terminal amino acid in the peptide sequence, which is composed of acidic or basic amino acid residues, such as Cys, Met, Tyr and Ser.

Dipeptidyl peptidase (DPP)-IV inhibitory activity

The DPP-IV inhibitory activity has been found in bovine hemoglobin, collagen and sheep collagen-derived peptides. Among them, the DPP-IV IC_{50} of sheep collagen is the lowest, which suggests the collagen-derived peptide has the strongest specific binding to DPP-IV and DPP-IV inhibitory activity. Furthermore, papain was the most effective protease to generate potent DPP-IV inhibitory peptides from bovine hemoglobin, and sheep skin. Notably, the sheepskin hydrolysate exhibited the highest DPP-IV inhibitory activity, achieving an *in vitro* inhibitory rate of 58%. A series of peptide sequences with DPP-IV inhibitory activities were identified, including GPAGPIGPV, GPAGPOGFPG, GPVG, FGPGP, APGGAP [16,24]. It is worth noting that the inhibitory activity is closely related to the presence of Pro residue at the C-terminal of peptide.

Antihypertensive activity

ACE-I inhibitory activity of peptides has been found in protein hydrolysates of meat by-products, such as blood and red blood cells. For example, Lafarga *et al.* found that after papain-treated hydrolysis, bovine hemoglobin had high ACE and renin inhibitory activities [25]. The values of ACE-I IC_{50} and renin IC_{50} were 0.19 mM and 7.09 mM. Compared with seaweed protein hydrolysate (IRLIIVLMPILMA) only having the renin-inhibiting activity, bovine hemoglobin with ACE-I and renin-inhibiting activity exhibited higher antihypertensive properties [25]. The ACE inhibitory activity of the obtained peptides (TPYPCV, VVYPWR, FLCT), generated through the hydrolysis of porcine red blood cells with pepsin and trypsin, resulted in an ACE IC50 value of 2.58 μL. In comparison to α-bovine hemoglobin in bovine blood, the extract from porcine red blood cells exhibits enhanced ACE inhibitory activity. Nevertheless, when contrasted with the ACE-targeting drug Lisinopril (IC50=0.2 nm), the ACE inhibitory activity of the porcine red blood extract is comparatively weak [26].

Antimicrobial peptides

Peptides derived from bovine hemoglobin and porcine serum albumin have been documented to demonstrate antibacterial properties. The antibacterial spectrum of bovine hemoglobin is broader than that of porcine serum albumin, exhibiting inhibition against two Gram-positive bacteria (*Micrococcus luteus* and *Listeria innocua*), and two Gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) [21]. The inhibitory effect of porcine serum albumin-derived peptides on *Bacillus cereus* has been identified. TSKYR, a pentapeptide obtained by hydrolysis of bovine hemoglobin by pepsin, exhibited good antibacterial activity (MIC,1.9 μM) [21]. Jin and co-workers found that porcine plasma albumin was hydrolyzed by trypsin to produce antibacterial peptides with a potent antibacterial effect against *Bacillus cereus* [27]. The antibacterial effects were as follows: at a sample dosage of 64 mg, the diameter of the antibacterial zone was 2.55 cm, surpassing the antibacterial effect of antimicrobial peptides (2.5 mg). Porcine placenta-derived peptides exhibited notable antibacterial efficacy against *S. aureus* and *E. coli*, with antibacterial zone diameters of 10.7 mm and 7.8 mm, respectively [28].

Anti-inflammatory activity

Nitric oxide (NO), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are commonly observed inflammatory factors. The prevention of excessive production of inflammatory factors can mitigate the risk of inflammatory diseases, such as atherosclerosis and metabolic syndrome. Bioactive peptides with inhibitory effects on inflammatory factors have been identified in both yak bone and bovine lung. Peptides, including GPAGPSGPAGK and GPSGPQGIR, produced by the combined enzymatic hydrolysis of yak bone using alcalase, neutrase and flavourzyme, can effectively regulate the NK-κB signaling pathway and NO production to inhibit the inflammatory response [29]. Bovine lung underwent hydrolysis with alcalase, and the resulting hydrolyzed products exhibited noteworthy anti-inflammatory activity in RAW264.7 macrophages. This activity was marked by a substantial reduction in the production of IL-6, IL-1β, and NO [30].

Other bioactivities

In addition to the aforementioned common biological activities, bioactive peptides also exhibit other bioactivities including tyrosinase inhibition, calcium-binding, anti-aging, and anti-fatigue activities. Tedeschi *et al.* reported that following papain hydrolysis, the hydrolysate derived from

calf fleshing meat demonstrated heightened tyrosinase inhibitory activity, reaching 55.6% at a 10% addition level [31]. This activity was comparable to the anti-tyrosinase activity observed in a substance extracted from *Scomber japonicus* [31], which can be used as a food preservative or as an additive in the production of food packaging. Choi *et al.* found that when the amount of protein hydrolysate obtained by thermal hydrolysis of porcine skin collagen was 0.5%, the inhibitory activity of tyrosinase was 33%, the inhibitory activity of collagenase was 49%, and the inhibitory rate of Elastase was 22% [32]. Sheep bone collagen-derived peptides (GPSGLPGERG and GAPGKDGVRG) obtained by the proteolytic action of alcalase and neutrase exhibited high calcium-binding capacity (89%), which can be used as calcium supplements in food and drugs.

Three dipeptide isomers of aspartic acid (Lα, Dα, Lβ), resulting from the oral administration of pig liver hydrolysate, exhibited anti-fatigue effects by activating AMPK in the liver at low concentrations [33]. In comparison to 0.1% ascorbic acid (1 mg/mL), the inhibition rates of collagenase and elastase were similar to that of ascorbic acid, with the exception of lower anti-tyrosinase activity. The hydrophobic amino peptide (Pep-NH_2) present in the protein hydrolysate of porcine liver, when administered through intraperitoneal injection to mice, demonstrated an enhancement in exercise activity after forced walking. Moreover, there are certain disease-related functional properties, such as the potential treatment of alcoholic liver disease and cardiovascular disease. The liver-protecting peptide isolated from porcine liver through alcalase-treated hydrolysates exhibited the capability to activate ethanol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH). The sequence NTLPHPTAP was found to bind to ALDH, and the formation of the complex ADH/ALDH-NTLPHPTAP extended the overall structure of the enzyme, facilitating its enhanced binding to the substrate [34].

Regulatory barriers to valorization, challenges and opportunities

Current regulations and restrictions related to meat by-products valorization

The threat of transmission of contagious diseases like swine fever, epidemics of foot and mouth disease, and spread of zoonotic infections, including TSEs (BSE, mad cow disease) while utilizing animal by-products have raised public health concerns. This has prompted various regulatory bodies, including the EU to implement restrictions and regulations on use, storage, disposal, export and import of the types and volumes of meat by-products (**Table 2**). In order to safeguard the

animal and human health, animal by-products that are classified under "Category 3" and in the lowest risk group are now permitted for food and feed applications (EU Regulation 1069/2009). The purpose is to prevent transmission of animal diseases, contaminants etc., that could occur in processed feed or valorized products, including bioactive peptides/protein hydrolysates having functional applications.

The functional and nutritional claims of bioactive peptides/protein hydrolysates are also governed by regulatory guidelines. In Europe, the dietary or therapeutics values of bioactive peptides are evaluated by the EC (EC 1924/2006 regulation) [35]. Before declaring any novelty; characterization of the peptides, animal and human trials, and other safety issues are harmonized and revealed by the EFSA. In United States, the functional claims are monitored by the FDA [36], and the marketed products must have a disclaimer that can be delivered by the U.S. Food and Drug Administration. However, for therapeutic/drug claim, FDA pre-approval is necessary. In Japan, validation of functional foods is governed by FOSHU and FNFC guidelines, framed in 1991 under JMHLW. After clinical verifications and obtaining legal approval, producers get the FOSHU label on their packaged products. In India, functional values of food products are monitored by the FSSAI under FSS Act, 2006. For this, food products need at least 15 years and maximum 30 years of safe and efficient usage in India and other countries, respectively. In China, bioactive peptide-based food supplements are legalized by the CFDA (http://www.sfdachina.com/info/88-1.htm), and products are marketed with the Blue Hat logo. Besides, prior permission is required before conducting *in vitro* and *in vivo* clinical trials, safety issues etc.

Opportunities for regulatory improvement and innovation

Different countries have their own laws and regulations for utilization of animal by products. These differences in processing, value addition, characterization, and marketing pose challenges for global trade **(Fig. 3)**. Various process and product innovations, characterizing valorized bioactive peptides are underway. Some meat derived peptides are bitter in taste [3], which is an issue for their commercial rejections. Innovative tools like iBitter-Fuse and VirtualTaste can identify and mask the bitterness of peptides by blocking bitter taste receptor activity [8]. Furthermore, use of endo- and exo-peptidases, γ-glutamyl transpeptidase from *Bacillus amyloliquefaciens*, or glycation of protein hydrolysates can reduce the bitterness to great extent [37,38]. Another innovative concept is *in silico* hydrolysis simulation model, which can help in

predicting known protein sequences and cleavage sites of chemicals or enzymes to extract new generation bioactive components [39]. Likewise, encapsulation of bioactive peptides can overcome the limitations of nutraceutical and commercial applications by improving the bioactivity, stability, solubility, food matrix interaction, sensory properties etc. [37,40].

Safety barriers to valorization

Microbial safety considerations: risks associated with meat by-products and control measures ensuring safety

Animal by-products or derived products, if contaminated and insufficiently processed, can be a source of microbial (Parasitic, bacterial and viral) hazards. In order to reduce the microbiological risks associated with animal by-products and protect environmental, animal and human health; microbial safety assessment is required at every stage along of the continuum from farm-to-fork [41]. Identification of potential hazards and implementation of control measures by combining physical and chemical methods, enforcing safety regulations and food safety standards can help in mitigating the risk of contamination. Routine screening of animal by-products, feed and bioactive peptides, by endorsement of traceability, is also a radical step to mitigate the epidemiological risks [42]. During processing of animal by-products, Hazard Analysis and Critical Control Point (HACCP) needs to be implemented at every step of production and processing to overcome the risks of microbiological hazards. Further, valorization of meat by-products should be restricted to materials belonging to Category 3 only, to mitigate the challenges of BSE and other specified risk materials [3].

Chemical contaminants and residues

Veterinary drugs, including antibiotics, anthelmintic and nonsteroidal anti-inflammatory drugs (NSAIDs) are often used to enhance production, and treat diseases in animals. However, the benefits of drug administration are not without associated risks, as the drug residues or metabolites may get accumulated within the tissues and meat byproducts of animals. Likewise, the source of chemical contaminants (pesticides, heavy metals, additives, toxins etc.) contracted by animals could be water, environment, feed and fodder.

As the goal is to produce safe and high-quality bioactive peptides, and functional ingredients, it is important to screen animal by-products through residue monitoring programs and implement quality control measures. First of all, the drug residues and chemical contaminants in animal by-products should be within the set tolerances or maximum residue limits set by regulatory bodies [43]. For this, regular screening and testing of samples to detect and quantify veterinary drug and chemical residues is needed. Samples not fulfilling the standards and guidelines set by regulatory bodies may be discarded, in order to ensure safety and quality of the products. Additionally, it is possible to significantly decrease residues and contaminant levels in animal products and by-products by regulating the usage of veterinary drugs (strictly following dose, duration, withdrawal periods), adopting good veterinary practices, reducing exposure to chemical contaminants, and offering high-quality feed and water to animals [44].

Monitoring and mitigation strategies

Control strategies should include adoption of Good Animal Husbandry Practices and waste management [45], screening and detection of potential hazards and contaminants through monitoring and surveillance programs. Further, proper use of veterinary drugs, adherence to withdrawal periods, monitoring of feed quality etc. is also recommended to minimize the emerging contaminants. It is mandatory to have toxicological investigations *in vitro* in both animal and human model systems before opening the marketing channels [46]. As synergistic/additive effects of bioactive peptides could be suppressed by other peptides, their reactive natures or mode of actions need to be addressed, before adding into any food formulations [47]. In this regard, polyphenols, if introduced could offer synergistic effect and solution by forming peptide-polyphenol complexes, enhancing the safety and functionality of peptides [48]. Furthermore, regulatory societies should come up with daily recommended intake levels of bioactive peptides and their dose response effectiveness on human health. Environment friendly advanced scientific *in silico* validation are required to mitigate these health-related threats.

Concluding remarks

From the above discussion, this paper highlighted the importance of meat by-products as a source of bioactive peptides and functional ingredients while depicting the research gaps and areas for further exploration from the regulatory and safety perspectives. Clearly, there is opportunity to

bring uniformity in evaluation of animal by-products, especially for bioactive peptides, through collaborative efforts of various stakeholders and policy makers: i) in developing a comprehensive regulatory framework, ii) updating food safety standards, and iii) implementing robust traceability systems and labeling requirements. Above all, collaboration among regulatory bodies, industry stakeholders, and research institutions is crucial in sharing knowledge to establish best practices in improving the safety of meat by-products valorization. Further, human clinical trials must be done to substantiate the health claims, before introduction of bioactive peptide-based products into the market. Finally, a successful valorization of animal by-products requires implementation of several key measures with proper monitoring and mitigation strategies, as these small fractions of peptides with lower molecular weight are prone to show cytotoxicity, allergenicity, mutagenicity etc.

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Mohammed Gagaoua: Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Arun K. Das:** Data curation, Investigation, Methodology, Writing – original draft. **Yu Fu:** Data curation, Investigation, Methodology, Visualization, Writing – original draft, **Amira Leila Dib:** Data curation, Investigation, Writing – review & editing. **Pramod Kumar Nanda:** Data curation, Investigation, Methodology, Writing – original draft.

Disclosure of competing interest

The authors declare that there are no conflicts of interest.

Data availability

No data was used for the research described in the article.

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* of special interest

** of outstanding interest

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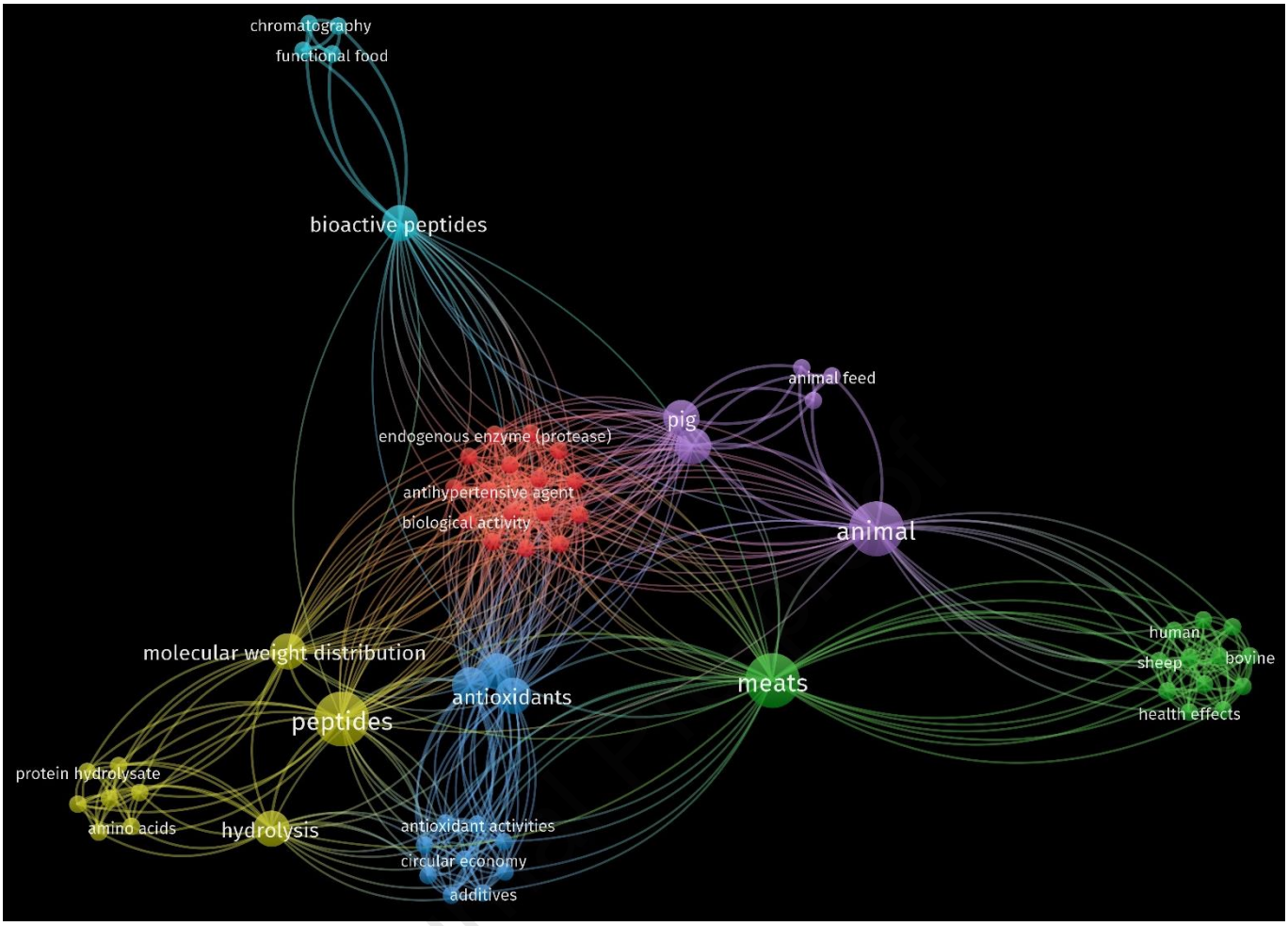

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533 **Fig. 1.** VoSViewer bibliometric map analysis of the 60 most frequent keywords from the articles
534 in the field of meat by-products valorization for the production of bioactive peptides and protein
535 hydrolysates (Scopus database search, 2020-2023). The size of the circles correlates with number
536 of occurrences. Keywords in the same cluster (same colour) frequently appear in the same articles
537 and create a specific group related to a thematic (e.g., source, bioactivity, methods, identified
538 characteristics, …etc). The lines highlight the degree of relationships among the keywords and
539 topics.

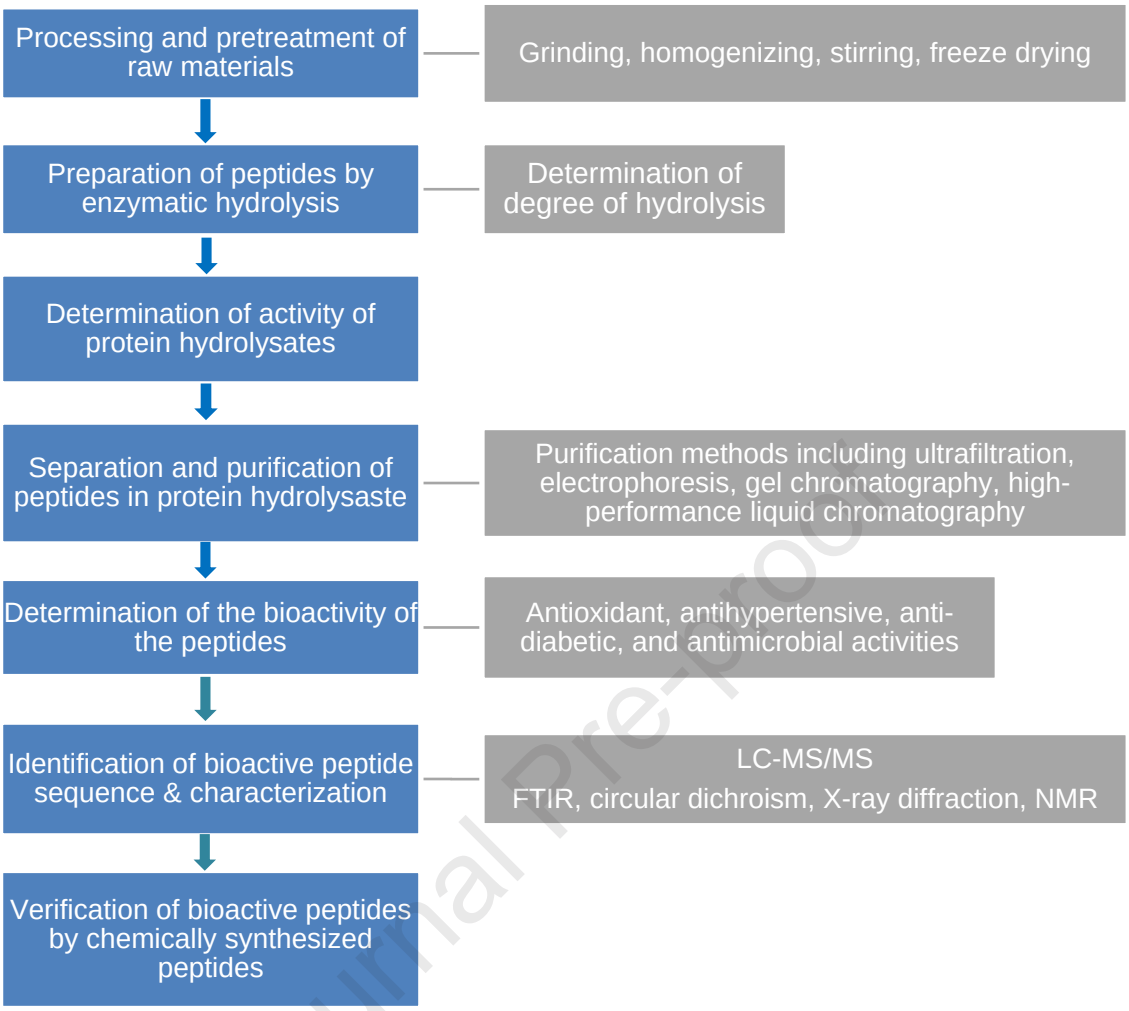
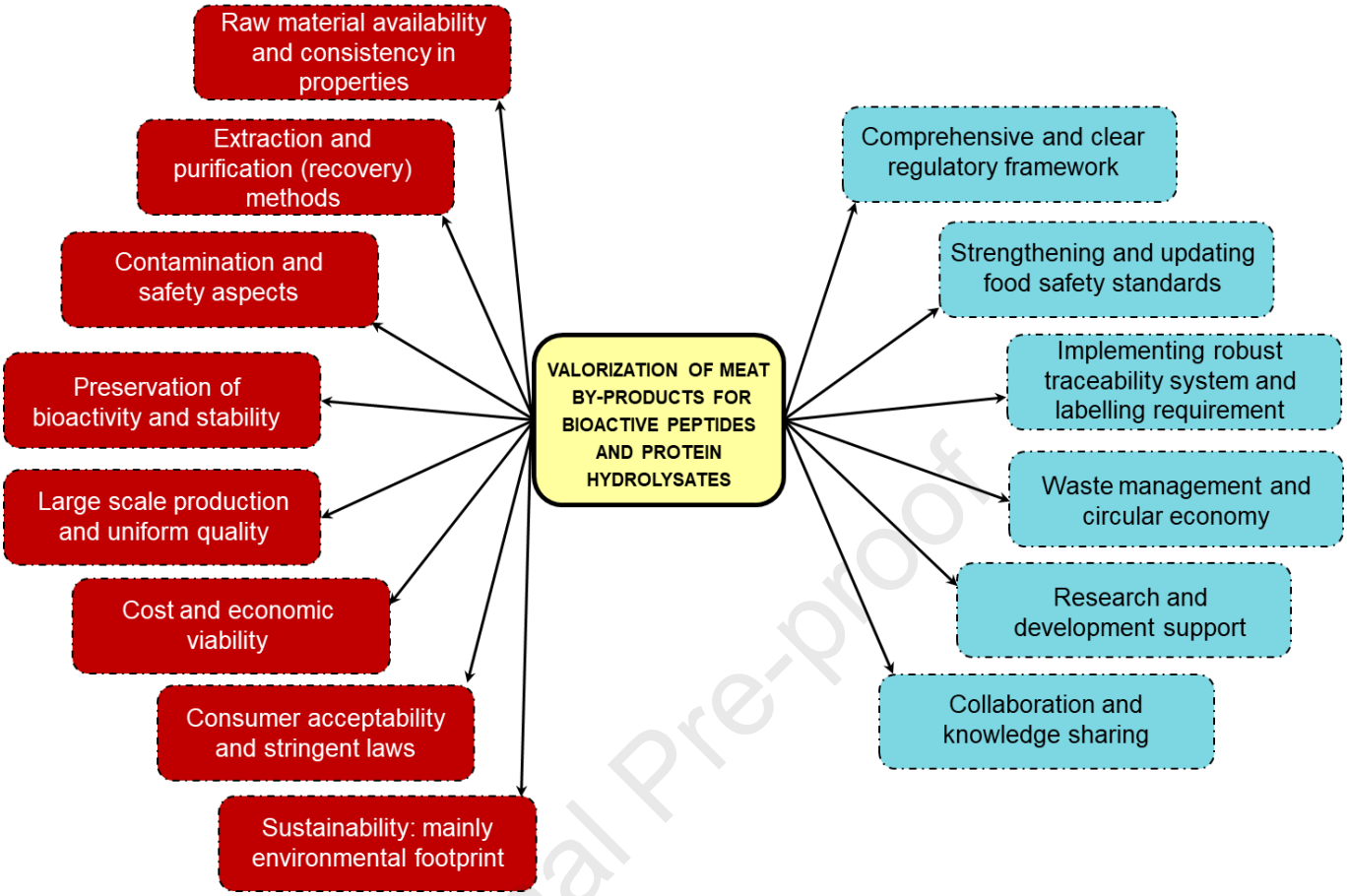


Fig. 2. Flow chart summarizing the main steps allowing the generation of bioactive peptides from meat by-products along with the methods and strategies used for their characterization. LC-MS/MS: Liquid Chromatography coupled to tandem Mass Spectrometry, FTIR: Fourier transform infrared spectroscopy, NMR: nuclear magnetic resonance.



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548 **Fig. 3.** Challenges and opportunities for valorization of meat by-products for bioactive peptides
549 and regulatory framework

Table 1. A non-exhaustive list of bioactive peptides from meat by-products.

Bioactivity	Peptide sequence	Source	Enzymes used and operating conditions [temperature in °C and time in min or hours]	Results	Refs.
Antioxidant capacity	N/A	Bovine bone collagen	Papain [65°C, 20h]	The amount of TBARS was 0.836 mg MDA/kg meat.	[14]
	TSKYR	Bovine hemoglobin	Pepsin [pH 3.5, 23°C, 30min]	The amount of TBARS was 1.75 mg MDA/kg meat.	[21]
	N/A	Bovine meat trimming	Papain [60°C, 2h]	The highest antioxidant activity was 3.22 g TEAC.	[31]
	N/A	Goat viscera	Hydrolysis using Alcalase [60°C] and further hydrolysis using brauzyn [70°C, 2h]	The scavenging rates of ABTS radical and DPPH radical were 70.65% and 42.24%, respectively.	[10]
	QTALVELLK, SLHTLFGDELCK, MPCTEDYLSLILNR	Sheep plasma	Alcalase [55°C, 6h]	The amount of FRAP was 0.335 mM $FeSO_4$/μg.	[49]
	LEDGLK, IDDVLK	Sheep abomasum	Papain [46°C, 3.8h]	The IC_{50} values of DPPH radical scavenging activity was 0.58 mg/mL.	[22]
	N/A	Porcine skin collagen	Hydrothermal processing [210°C, 2100kPa, 10min]	The scavenging activity of ABTS free radical reached 86% at 5 mg/Ml. The reducing power was 0.269 at 700 nm at 10 mg/mL.	[32]
	SY, PN, GS, KP, AOHR	Porcine bone collagen	Papain [55°C, 3h, 1:1(w/w)]	The DPPH scavenging activity reached 62.9% at 50 mg/mL.	[50]
	YDQLPEPRKPIE	Porcine blood	Hydrolysis using papain Pepsin [pH 2, 37°C, 1h] and further hydrolysis using trypsin [pH 7.0, 37°C, 2h]	The HRAS value was 66.89%, RAS value was 89.37%, and DPPH free radical scavenging rate was 38.32% at 5%. The cells exhibited the highest activity of 91.44% (The concentration of H_2O_2 was 700mM) at 0.1 mg/mL.	[23]
	HLDYYLGK DTYIRQPW MYPGIAD	Duck meat (liver)	Enzyme hydrolysis using alcalase (pH 8, 50◦C) papain (pH 7, 50◦C), and neutrase (pH 7, 50◦C)	The ABTS radicals-scavenging activities of HLDYYLGK, DTYIRQPW, and MYPGIAD had lower IC50 values than glutathione (GSH) group, with being 29.13 ±	[51]

				0.49μM, 36.23 ± 1.86μM, and 26.40 ± 0.59μM, respectively	
DPP-IV inhibitory activity	GPVG, FGPGP, APGGAP, GPPGPT, GPVGPPG	Bovine collagen	Papain and Protemax [50℃, 4h]	The lowest IC_{50} value of DPP-IV was 3.04 mg/mL.	[16]
	HR, YR, HLP	Bovine hemoglobin	Papain [65℃, 24h]	The lowest IC_{50} value of DPP-IV was 0.99 mg/mL.	[25]
	GPAGPIGPV, GPAGPOGFPG	Sheep skin collagen	Alcalase and Neutrase [37℃, 10min]	The lowest IC_{50} value of DPP-IV was 67.12 μg/mL.	[24]
Antihypertensive activity	HR, YR, HLP	Bovine hemoglobin	Papain [65℃, 24h]	The lowest IC_{50} of ACE-I was 0.19 mM, and the IC_{50} of renin was 7.09 mM.	[25]
	TPYPCV, VVYPWR, FLCT	Red blood cells	Pepsin [pH 2, 37℃, 2h] and Trypsin [pH 7.5, 37℃, 2h]	The lowest IC_{50} value of ACE-I was 2.58 Mm.	[26]
	N/A	Porcine liver	Autolysis [pH 4.8]	The IC_{50} value of ACE-I was 0.81g/L.	[52]
Antimicrobial activities	TSKYR	Bovine hemoglobin	Pepsin [pH 3.5, 23℃, 30min]	MIC were between 1 and 9 μM against four tested strain: *Micrococcus luteus, Listeria innocua, Escherichia coli and Salmonella enteritidi.*	[21]
	N/A	Porcine blood	Trypsin [pH 7, 50℃, 2h]	When the addition level was 64 mg, the hydrolysates have a great against for pathogenic microorganisms (*Bacillus cereus*). The inhibitory zone diameter was 2.55 cm.	[27]
	N/A	Porcine placenta	Papain [pH 6.5, 50℃, 20 min]	The inhibitory zone diameter was 10.7mm for *Staphylococcus aureus* and 7.8mm for *Escherichia coli.*	[28]
Anti-inflammatory activities	GPAGPSGPAGK, GPAGPSGPAGKDGR, GPSGPQGIR, GPAGPQGPR, GEAGPAGPAGPAGPR, GEGGPQGPR	Yak bone collagen	Hydrolysis using a mixture of alcalase, neutrase and flavourzyme [55℃, 4h]	When the dosage of peptide was 100 nmol/mL, the inhibition rate of 1L-1β reached 88.95%. The inhibition rates of TNF-α, IL-6 and NO were 60.23%, 63.98% and 60.11%.	[29]

	N/A	Bovine lung	Hydrolysis using papain [pH 6.5 65℃] and further hydrolysis using pepsin [pH 2.0 37℃] and alcalase [pH 9.5, 60℃, 24h]	When the hydrolysate concentration was 0.05%, IL-6 decreased by 53% and IL-1β decreased by 52%.	[30]
Anti-tyrosinase activity	N/A	Bovine meat trimming	Papain, 60℃, 2h	The inhibitory activity of tyrosinase was 55.6% at 10%.	[31]
	N/A	Porcine collagen	Hydrothermal processing [210℃, 2100 kPa, 10 min]	The inhibition rate of tyrosinase activity was 33% at 0.5%.	[32]
Calcium supplement	GPSGLPGERG, GAPGKDGVRG	Sheep bone collagen	Alcalase [50℃, 2h] and neutrase [50℃, 2h]	The calcium binding rate was 89%.	[15]
Anti-aging activity	N/A	Porcine collagen	Hydrothermal processing [210℃, 2100 kPa, 10 min]	At the concentration of 0.5%, the inhibition rates of tyrosinase, collagenase and elastase were 33%, 48.98% and 22.22%.	[32]

Table 2. Summary of the European Union (EU)/USDA/FSSAI regulations on safety aspects of animal by-products and derived products.

Regulations	**General Principles**
(EC) TSE Regulation No 999/2001	Total ban on using any remains of animals (by-products) in feed for livestock
EC Regulation No 852/2004	Sets out the hygiene requirements with respect to storage, handling, disposal/ elimination of all food waste, non-edible by-products and refuse
EC Regulation No 183/2005	Feed business operators, other than primary producers, are required to store and transport feed under certain hygienic conditions.
Regulation (EC) No 1069/2009, and legislation (Regulation (EU) No 142/2011)	Provides definition of animal by-products and categories (1, 2 and 3), requirements for compulsory treatment processes, permitted options for disposal, storage and labelling, transport, trade or future use of animal by-products Category 1: Material with high specific risk, as well as animal by-products containing some specifies substances and environmental contaminants. Category 2: Animal by-products with high risk, infected or contaminated carcasses, as well as materials declared unfit for human consumption Category 3: Includes inedible and free from infection carcass materials, as well as animal materials that are fit but not intended for human consumption for commercial reasons
Commission Decision 2009/719/EC	Revised monitoring programs allowing member States to test for the presence of TSEs in specific target groups of animals such as emergency slaughtered animals and fallen stock
Regulation (EU) 68/2013, Preambles 2 to 5	Feed ingredients should comply with the applicable restrictions for chemical and microbiological safety
Commission Regulation (EU) No 1924/2006, No 353/2008, No 1169/2009	EFSA's regulation on nutrition and health claims: functional food products need to be justified scientifically through clinical trials
Regulation (EU) 2021/1372 amending Regulation (EC) No 999/2001	Use of PAPs of poultry origin in pork feed and pig PAPs in poultry feed, use of ruminant collagen and gelatin in feed for non-ruminant farmed animals
FDA - Code of Federal Regulations (CFR) 21 (part 507)	Facilities that are not producing human food but are producing by-products for use as animal food are required to register and comply with all of 21 CFR part 507, unless they meet the criteria for an exemption
Food Safety and Standards (Food Product Standards and Food Additives) Amendment Regulations, 2020 under sub-regulation 2.5.2	Milk and meat producing animals except poultry, pig and fish shall not be fed with feed containing meat or bone meal including internal organs, blood meal and tissues of bovine or porcine origin materials except milk and milk products

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: