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# Meat by-products as a source of bioactive peptides and functional ingredients: Regulatory and safety barriers to valorization

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1 **Meat by-products as a source of bioactive peptides and functional ingredients: Regulatory**  
2 **and safety barriers to valorization**

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12

13

14 **Abstract**

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

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

## 29 Introduction

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

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

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

## 69 **Main strategies for valorization of meat by-products**

### 70 *Generation and extraction methods of bioactive peptides*

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

80 The main preparation processes of bioactive peptides are illustrated in **Fig. 2**, which include raw  
81 material pretreatment, enzymatic hydrolysis, separation and purification, bioactivity evaluation,  
82 peptide identification and activity verification. Firstly, raw material pretreatment involves  
83 procedures, such as bone grinding, freeze-drying of viscera, and homogenization. Additional  
84 pretreatment methods include blood centrifugation and decolorization. These processes contribute  
85 to the degradation of structure of the samples, hence facilitating the entry of enzymes and thereby  
86 initiating the protein hydrolysis. Enzymatic hydrolysis typically occurs in both batch reactors and  
87 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, electro dialysis, 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. Electro dialysis 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].

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

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

## 134 **Diversity of peptide bioactivities in meat by-products**

### 135 *Antioxidant activity*

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

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

#### 151 *Dipeptidyl peptidase (DPP)-IV inhibitory activity*

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

#### 161 *Antihypertensive activity*

162 ACE-I inhibitory activity of peptides has been found in protein hydrolysates of meat by-  
163 products, such as blood and red blood cells. For example, Lafarga *et al.* found that after papain-  
164 treated hydrolysis, bovine hemoglobin had high ACE and renin inhibitory activities [25]. The  
165 values of ACE-I IC<sub>50</sub> and renin IC<sub>50</sub> were 0.19 mM and 7.09 mM. Compared with seaweed protein  
166 hydrolysate (IRLIIVLMPILMA) only having the renin-inhibiting activity, bovine hemoglobin with  
167 ACE-I and renin-inhibiting activity exhibited higher antihypertensive properties [25]. The ACE  
168 inhibitory activity of the obtained peptides (TPYPCV, VVYPWR, FLCT), generated through the  
169 hydrolysis of porcine red blood cells with pepsin and trypsin, resulted in an ACE IC<sub>50</sub> value of  
170 2.58 μL. In comparison to α-bovine hemoglobin in bovine blood, the extract from porcine red blood  
171 cells exhibits enhanced ACE inhibitory activity. Nevertheless, when contrasted with the ACE-  
172 targeting drug Lisinopril (IC<sub>50</sub>=0.2 nm), the ACE inhibitory activity of the porcine red blood  
173 extract is comparatively weak [26].

#### 174 *Antimicrobial peptides*



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

#### 188 *Anti-inflammatory activity*

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

#### 200 *Other bioactivities*

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



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

213 Three dipeptide isomers of aspartic acid ( $L\alpha$ ,  $D\alpha$ ,  $L\beta$ ), resulting from the oral administration of  
214 pig liver hydrolysate, exhibited anti-fatigue effects by activating AMPK in the liver at low  
215 concentrations [33]. In comparison to 0.1% ascorbic acid (1 mg/mL), the inhibition rates of  
216 collagenase and elastase were similar to that of ascorbic acid, with the exception of lower anti-  
217 tyrosinase activity. The hydrophobic amino peptide (Pep-NH<sub>2</sub>) present in the protein hydrolysate  
218 of porcine liver, when administered through intraperitoneal injection to mice, demonstrated an  
219 enhancement in exercise activity after forced walking. Moreover, there are certain disease-related  
220 functional properties, such as the potential treatment of alcoholic liver disease and cardiovascular  
221 disease. The liver-protecting peptide isolated from porcine liver through alcalase-treated  
222 hydrolysates exhibited the capability to activate ethanol dehydrogenase (ADH) and acetaldehyde  
223 dehydrogenase (ALDH). The sequence NTLPHPTAP was found to bind to ALDH, and the  
224 formation of the complex ADH/ALDH-NTLPHPTAP extended the overall structure of the  
225 enzyme, facilitating its enhanced binding to the substrate [34].

## 226 **Regulatory barriers to valorization, challenges and opportunities**

### 227 *Current regulations and restrictions related to meat by-products valorization*

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

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

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

### 253 *Opportunities for regulatory improvement and innovation*

254 Different countries have their own laws and regulations for utilization of animal by products.  
255 These differences in processing, value addition, characterization, and marketing pose challenges  
256 for global trade (**Fig. 3**). Various process and product innovations, characterizing valorized  
257 bioactive peptides are underway. Some meat derived peptides are bitter in taste [3], which is an  
258 issue for their commercial rejections. Innovative tools like iBitter-Fuse and VirtualTaste can  
259 identify and mask the bitterness of peptides by blocking bitter taste receptor activity [8].  
260 Furthermore, use of endo- and exo-peptidases,  $\gamma$ -glutamyl transpeptidase from *Bacillus*  
261 *amyloliquefaciens*, or glycation of protein hydrolysates can reduce the bitterness to great extent  
262 [37,38]. Another innovative concept is *in silico* hydrolysis simulation model, which can help in

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

## 267 **Safety barriers to valorization**

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

270 Animal by-products or derived products, if contaminated and insufficiently processed, can be a  
271 source of microbial (Parasitic, bacterial and viral) hazards. In order to reduce the microbiological  
272 risks associated with animal by-products and protect environmental, animal and human health;  
273 microbial safety assessment is required at every stage along of the continuum from farm-to-fork  
274 [41]. Identification of potential hazards and implementation of control measures by combining  
275 physical and chemical methods, enforcing safety regulations and food safety standards can help in  
276 mitigating the risk of contamination. Routine screening of animal by-products, feed and bioactive  
277 peptides, by endorsement of traceability, is also a radical step to mitigate the epidemiological risks  
278 [42]. During processing of animal by-products, Hazard Analysis and Critical Control Point  
279 (HACCP) needs to be implemented at every step of production and processing to overcome the  
280 risks of microbiological hazards. Further, valorization of meat by-products should be restricted to  
281 materials belonging to Category 3 only, to mitigate the challenges of BSE and other specified risk  
282 materials [3].

283 *Chemical contaminants and residues*

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

290 As the goal is to produce safe and high-quality bioactive peptides, and functional ingredients, it  
291 is important to screen animal by-products through residue monitoring programs and implement  
292 quality control measures. First of all, the drug residues and chemical contaminants in animal by-  
293 products should be within the set tolerances or maximum residue limits set by regulatory bodies  
294 [43]. For this, regular screening and testing of samples to detect and quantify veterinary drug and  
295 chemical residues is needed. Samples not fulfilling the standards and guidelines set by regulatory  
296 bodies may be discarded, in order to ensure safety and quality of the products. Additionally, it is  
297 possible to significantly decrease residues and contaminant levels in animal products and by-  
298 products by regulating the usage of veterinary drugs (strictly following dose, duration, withdrawal  
299 periods), adopting good veterinary practices, reducing exposure to chemical contaminants, and  
300 offering high-quality feed and water to animals [44].

### 301 *Monitoring and mitigation strategies*

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

### 315 **Concluding remarks**

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

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

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

### 339 **Disclosure of competing interest**

340 The authors declare that there are no conflicts of interest.

### 341 **Data availability**

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

### 343 **References**

344 Papers of particular interest, published within the period of review, have been highlighted as:

345 \* of special interest

346 \*\* of outstanding interest

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356 *possible applications of bioactive peptides derived from different meat sources and the by-products*  
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360 \* *This is among the first comprehensive reviews that addressed the bioactive peptides identified in*  
361 *muscle proteins and by-products generated during the processing of meat. The paper looks at the*  
362 *isolation, enrichment and characterization strategies that have been employed to date to generate*  
363 *bioactive peptides and the potential future applications of these peptides in functional foods for the*  
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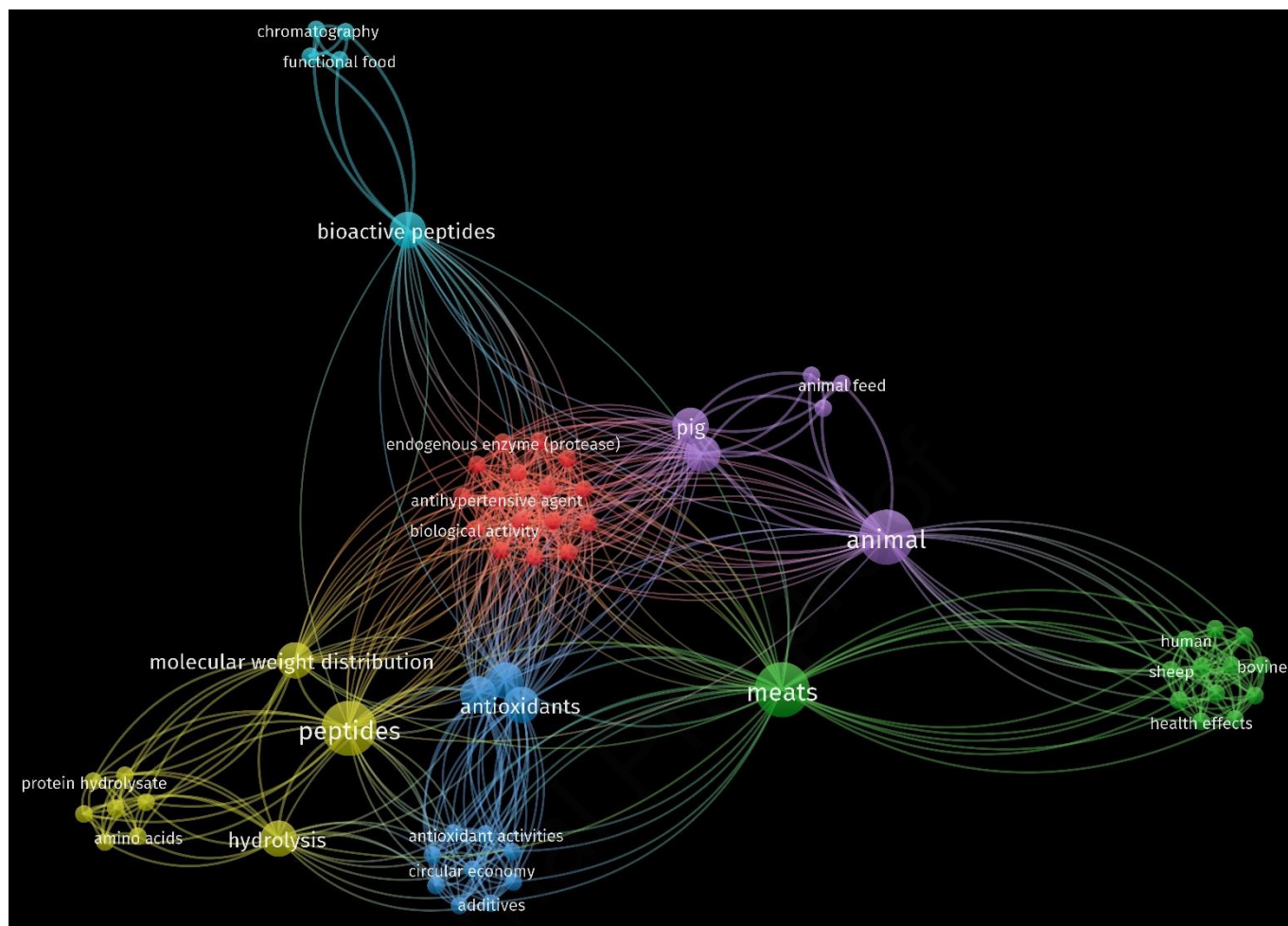
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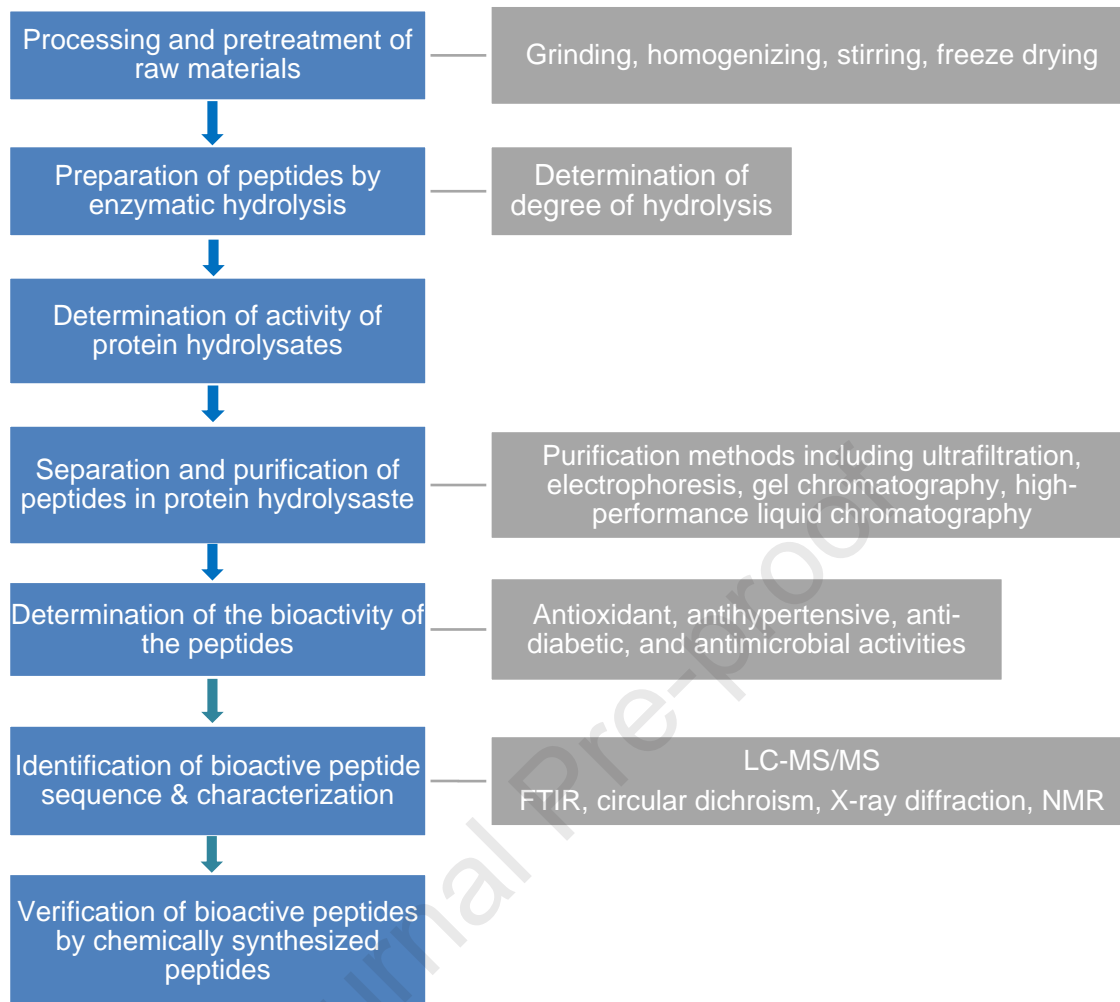
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533 **Fig. 1.** VoSViewer bibliometric map analysis of the 60 most frequent keywords from the articles  
 534 in the field of meat by-products valorization for the production of bioactive peptides and protein  
 535 hydrolysates (Scopus database search, 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.

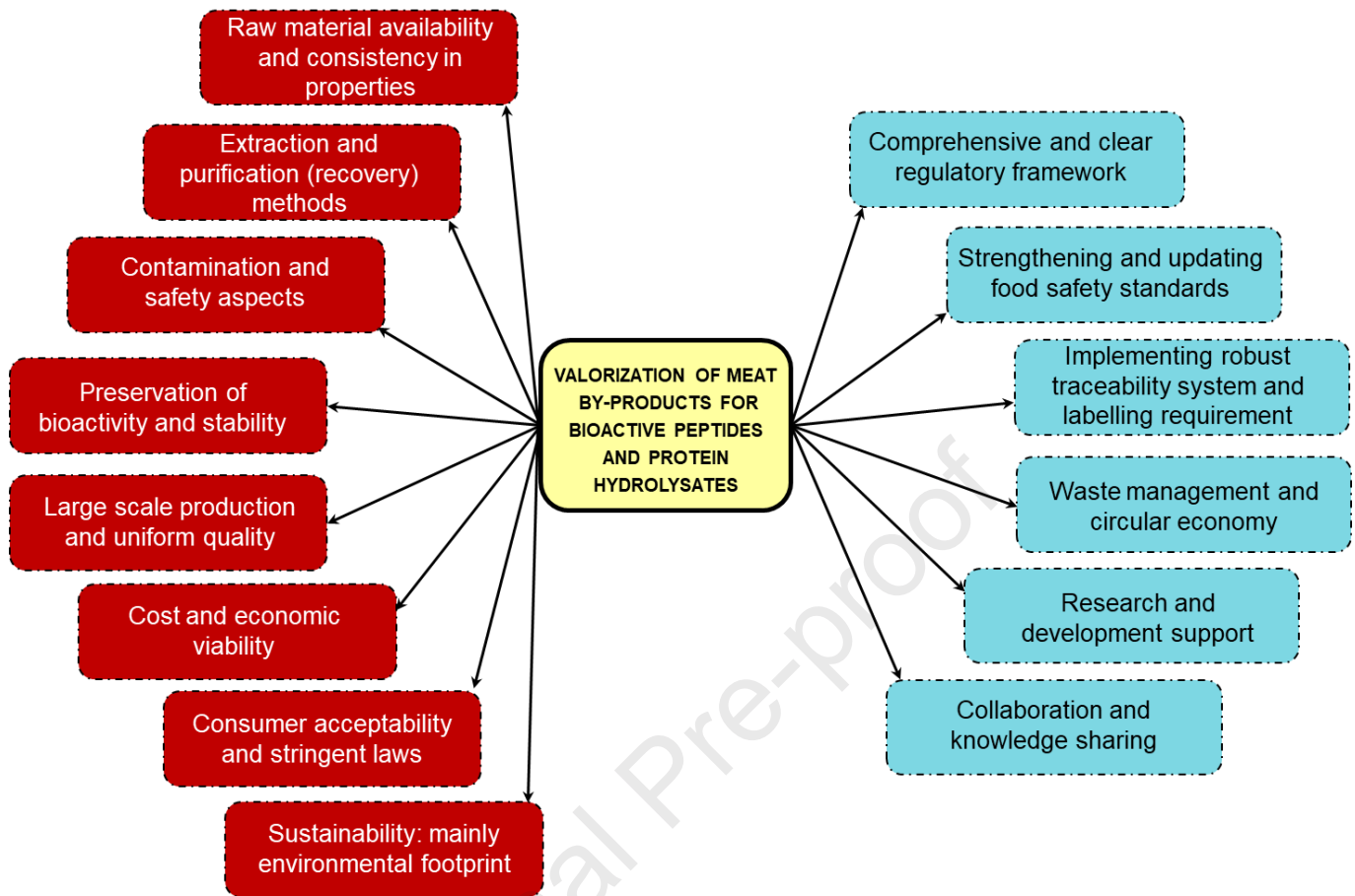


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541 **Fig. 2.** Flow chart summarizing the main steps allowing the generation of bioactive peptides from  
 542 meat by-products along with the methods and strategies used for their characterization. LC-  
 543 MS/MS: Liquid Chromatography coupled to tandem Mass Spectrometry, FTIR: Fourier transform  
 544 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 <sub>4</sub> /μg.	[49]
	LEDGLK, IDDVLK	Sheep abomasum	Papain [46°C, 3.8h]	The IC <sub>50</sub> 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 <sub>2</sub> O <sub>2</sub> 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 IC <sub>50</sub> values than glutathione (GSH) group, with being 29.13 ±	[51]

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

	N/A	Bovine lung	Hydrolysis using papain [pH 6.5 65°C] and further hydrolysis using pepsin [pH 2.0 37°C] and alcalase [pH 9.5, 60°C, 24h]	When the hydrolysate concentration was 0.05%, IL-6 decreased by 53% and IL-1 $\beta$ decreased by 52%.	[30]
Anti-tyrosinase activity	N/A	Bovine meat trimming	Papain, 60°C, 2h	The inhibitory activity of tyrosinase was 55.6% at 10%.	[31]
	N/A	Porcine collagen	Hydrothermal processing [210°C, 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°C, 2h] and neutrase [50°C, 2h]	The calcium binding rate was 89%.	[15]
Anti-aging activity	N/A	Porcine collagen	Hydrothermal processing [210°C, 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]

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

<b>Regulations</b>	<b>General Principles</b>
(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 specific 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

3

**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:

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