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POULTRY PROCESSING BY-PRODUCTS AS POTENTIAL SOURCES OF BIOACTIVE PEPTIDES

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Abstract

Poultry meat is characterized by its good nutritive value in terms of protein content with high biological value, as well as relatively low fat content composed of a higher proportion of unsaturated fatty acids. The poultry-processing industry is one of the fastest-growing food sectors. In this industry with such a vast production volume, the generation of a considerable amount of waste is inevitable. Most of these waste materials are extremely rich in organic compounds and nutrient components. Therefore, their evaluation through different strategies is a matter of significant importance. Poultry by-products are recognized as remarkable protein sources facilitating the production of bioactive peptides at an industrial scale. These by-products are converted into protein hydrolysates mostly by enzymatic hydrolysis to provide the release of bioactive peptides. Various by-products obtained during poultry processing such as blood, skin, feathers, viscera, liver and feet have been reported to possess plenty of bioactivities after hydrolysis. The highlighted bioactive features of those sources mainly include antioxidant, antihypertensive and antimicrobial functions. Consequently, the poultry-processing industry presents a burgeoning opportunity for the valorization of by-products rich in bioactive peptides. As the demand for sustainable practices and novel functional ingredients continues to rise, future studies exploring the diverse bioactivities and applications of the peptides derived from poultry by-products are essential for maximizing their potential contribution to human health and the food industry.

Keywords: *biopeptide, antioxidant peptides, antihypertensive peptides, antimicrobial peptides, chicken processing, poultry waste, food waste valorization*

1. INTRODUCTION

Over the past few decades, there has been a notable increase in both the production and consumption of poultry meat on a global scale. Projections from industrial sources indicate a sustained upward trend in production levels in the following years, even the demand for poultry is expected to double by the end of 2050 (Franzo et al. 2023). The large scale of poultry production results in the generation of substantial quantities of by-products. These by-products, whether edible or inedible, may constitute up to 30% of the total production output, thereby posing significant challenges related to environmental impacts and associated disposal expenses (Ozturk-Kerimoglu et al. 2023). For this reason, there is a pressing need for effective strategies aimed at recovering these poultry residues to support circular bioeconomy. One of the most useful strategies for the valorization of poultry by-products is to convert them into protein hydrolysates to obtain value-added products (Angulo & Carmen 2023).

Bioactive peptides are defined as short peptide fragments comprising two to twenty amino acids that could positively modulate human physiological functions after consumption at optimal amounts (Ashaolu et al. 2023, Bechaux et al. 2019). Such peptides can be generated during the processing of meat products or from precursor meat by-products by treatments such as enzymatic/chemical hydrolysis or microbial fermentation (Lafarga & Hayes 2014). Since the peptide sequences are hidden in the whole protein chain, the technology to promote an adequate peptide release is the key factor determining the final yield (Xing et al. 2021). Those peptides possess diverse functions such as antioxidant, antimicrobial, immunomodulatory, anticancer, antihypertensive, and antithrombotic features as well as enhancing sensory quality, rheological properties, and shelf life of food products (Lafarga & Hayes 2014, Bhat et al. 2015, Cheng et al. 2016, Dullius et al. 2020, Toldrá et al. 2020) as could be seen in Fig. 1. In the present paper, it was objected to review the potential impacts of various poultry by-products as sources of bioactive peptides.

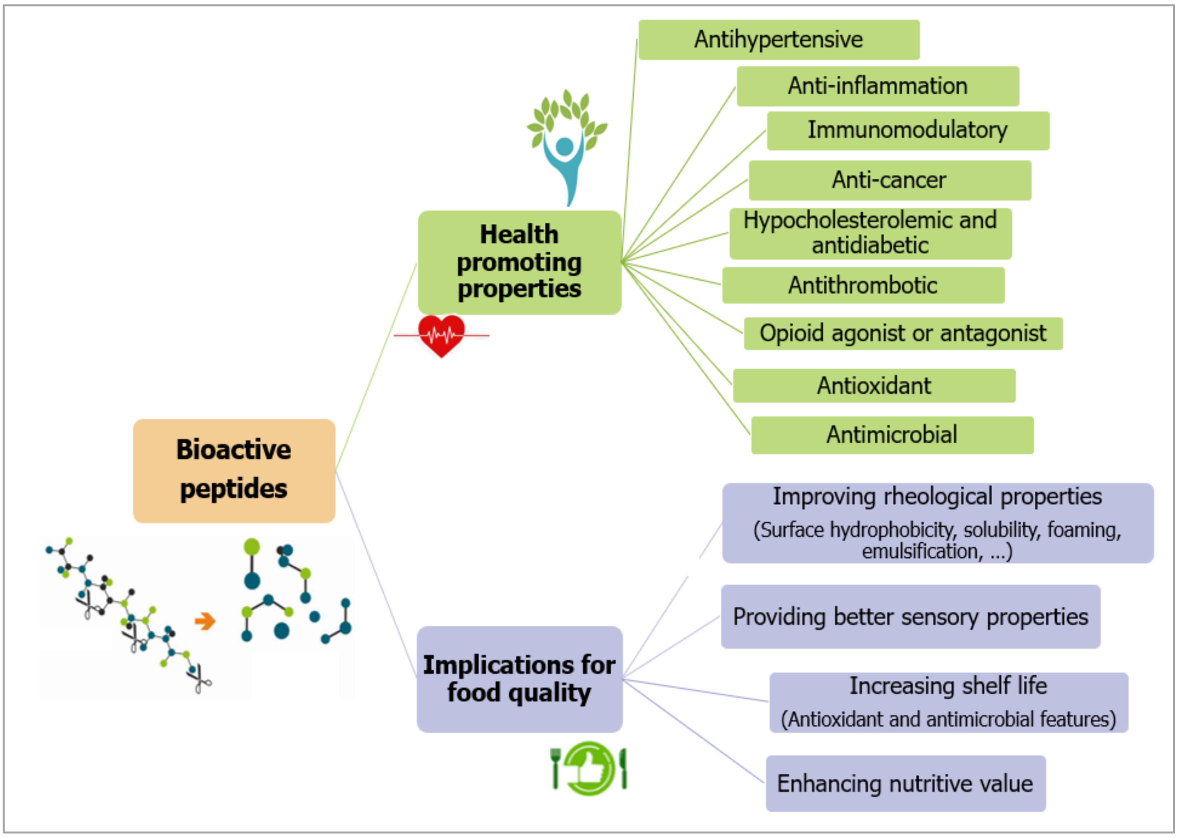

Fig. 1. Beneficial features of bioactive peptides
(Adapted from Lorenzo et al. 2018, Karami & Akbari-Adergani 2019, Toldrá et al. 2020).

2. CLASSIFICATION OF POULTRY BY-PRODUCTS

In broiler slaughterhouses, various by-products such as heads, blood, bone, trimmings, skin, adipose tissues, feet, and internal organs are generated in large amounts which are typically not consumed as food. Those parts that cannot be evaluated as food are often disposed of and removed without further processing; however, the majority of those by-products are excellent protein sources (Lasekan et al. 2013, Gál et al. 2020). According to EU legislation, the by-products of poultry are typically grouped into three main categories depending on the risk level for transmitting pathogens and toxic materials. These categories are presented in Table 1. Category-3 covers the by-products that could be used for certain purposes such as skin, feathers, heads, feet, horns, and hoof. Category-2 by-products are regarded as high-risk products, but they can be used as feedstock for composting, biogas, and energy generation. On the side, the by-products that belong to Category-1 need to be buried or incinerated at designated sites (Lasekan et al. 2013). Since poultry by-products are stated as valuable protein sources, research on peptides in poultry hydrolyzed proteins can be promising in the field of nutrition and medicine while providing health benefits (Etemadian et al. 2021).

EU classification	Use of the by-product
Category-3	Livestock feed
Category-3	Pet food
Category-3	Aqua feed
Category-3	Cosmetic products
Category-2 and Category-3	Compost
Category-2 and Category-3	Production of biogas
Category-1, Category-2 and Category-3	Production of thermal and electrical energy
Category-1, Category-2 and Category-3	Production of biofuel

Table 1. Chicken by-products and their corresponding categories defined by the Commission of the European Communities Regulation (EC) (Lasekan et al. 2013).

3. MAIN BIOACTIVITIES OF HYDROLYSATES AND/OR PEPTIDES DERIVED FROM POULTRY BY-PRODUCTS

Current studies on the bioactivity potentials of protein hydrolysates obtained from various poultry by-products are summarized in Table 2. Protein hydrolysates derived from chicken by-products such as blood proteins, feathers, skin, viscera, liver and feet, as well as peptides identified within these hydrolysates, have been reported to exhibit significant bioactivity characteristics. As is seen from the related table, the beneficial features of the hydrolysates/peptides derived from those by-products mostly focused on antioxidant activity. Besides, antihypertensive, antimicrobial and antidiabetic functions were also reported. These bioactive properties will be elucidated in subsequent sections.

3.1. Antioxidant activity

When reactive oxygen species (ROS) are generated in excess, the oxidative stress phenomenon is initiated and thereby these ROS can oxidize lipids, proteins, and nucleic acids that could further result in cell damage and apoptosis (Lafarga & Hayes 2014). The damage in the biomacromolecules is associated with serious diseases including hypertension, cancer, diabetes, neurodegenerative disorders, heart disease, stroke, as well as with aging (Liu et al. 2016). The consumption of antioxidant compounds in the diet and the search for potential antioxidant sources are still issues of high priority to meet the consumer demand for healthier foods. Although numerous studies have been carried out regarding the antioxidant roles of plant polyphenol derivatives among many different natural sources, research on the antioxidant activities of peptides derived from food proteins has been more recently initiated (Fukada et al. 2016, Lorenzo et al. 2018).

Antioxidant characteristics of the proteins are formed depending on their complex interactions with lipids, oxidation catalysts, and free radicals. Thus, proteins differ from other food-derived antioxidants since they have diverse functional properties that prevent different metabolic pathways of oxidation (Abeyrathne et al. 2018). It is possible to obtain many different antioxidative peptides from different animal sources as well as their by-products. Due to their ability to scavenge free radicals and to chelate metal ions by inhibiting electron migration and hydrogen atom transfer, inhibitory potentials of those peptides on enzymatic and non-enzymatic peroxidation of lipids and essential fatty acids have been documented (Bhat et al. 2015, Cheng et al. 2016, Fu et al. 2017, Dullius et al. 2020).

By-product	Method for hydrolysis	Bioactive feature	Reference
Liver	Fermentation and enzymatic hydrolysis	Antioxidant Antibacterial	Chakka et al. (2015)
Blood plasma and blood cell proteins	Enzymatic hydrolysis with Alcalase	Antioxidant	Cheng et al. (2016)
Viscera	Enzymatic hydrolysis with different commercial proteases (Flavourzyme, Alcalase, and Neutrase)	Antihypertensive	de Souza et al. (2017)
Feather	Microbial conversion during submerged cultivation using three feather-degrading *Bacillus* strains (CL18, CL33A, CL14)	Antioxidant Antihypertensive Antidiabetic	Callegaro et al. (2018)
Skin	Enzymatic hydrolysis with Alcalase, Pronase E and Collagenase enzymes combined with ultrafiltration	Antioxidant	Sarbon et al. (2018)
Foot	Enzymatic hydrolysis with Protamex	Antihypertensive	Bravo et al. (2019)
Skin	Enzymatic hydrolysis with Elastase	Antioxidant	Nadalian et al. (2019)
Combs and wattles	Enzymatic hydrolysis with Alcalase	Antioxidant	Bezerra et al. (2020)
Feather	Enzymatic hydrolysis with different proteases (Neutrase, Alcalase, and Flavourzyme) at varying concentrations	Antioxidant	Jeampakdee et al. (2020)
Feather	Microbial hydrolysis by *Bacillus amyloliquefaciens* KB1	Antioxidant	Prajapati et al. (2021)
Foot	Enzymatic hydrolysis with different commercial enzymes (Alcalase, Flavourzyme and Protana Prime)	Antioxidant	Ozturk-Kerimoglu et al. (2023)

Table 2. Studies conducted on obtainment and characterization of protein hydrolysates/bioactive peptides from different chicken by-products.

Although the exact mechanism of the antioxidant activity of the biopeptides isolated from meat and meat by-products has not been fully discovered yet, some common reasons for possessing antioxidant activity are pointed out in the studies that could be summarized as follows:

- Antioxidant peptides identified commonly show a size of 4-16 amino acids, and their molecular weight is approximately 400-2000 Da.
- The amino acid sequence is emphasized to play a vital role in antioxidant activity.
- Aromatic amino acids including tyrosine (Tyr), histidine (His), tryptophan (Trp) and phenylalanine (Phe) are mentioned to have the ability to donate protons to the electron-deficient radicals that could lead to radical scavenging. His-containing peptides are addressed to indicate

a high antioxidant capacity due to the imidazole ring in the R group with hydrogen donating and metal ion-chelating ability. In addition, the position of His at the *N*-terminus is emphasized to contribute to the antioxidant capacity, and Trp and Tyr residues at the C-terminus are associated with strong radical scavenging ability.

- Hydrophobic amino acids that consist of alanine (Ala), isoleucine (Ile), leucine (Leu), proline (Pro), methionine (Met), Phe, Tyr and Trp could increase the presence of peptides at the water-lipid interface and facilitate access to scavenge free radicals from the lipid phase.
- Acidic (aspartic acid-Asp and glutamic acid-Glu) and basic (arginine-Arg, lysine-Lys and His) amino acids use carbonyl and amino groups in the side chain which function as metal ions chelators.
- Cysteine also contributes to antioxidant capacity which is attributed to the sulfhydryl in the R groups.
- Some researchers also stated that spatial conformation of the peptides might lead to synergistic or antagonist effects when compared with the role of amino acids. Further, the secondary structure of the peptides is associated with enhanced antioxidant capability since it affects binding characteristics (Liu et al. 2016, Toldrá et al. 2020, Ozturk-Kerimoglu et al. 2023).

It has been reported that hydrolysates obtained from various poultry by-products could exhibit significant antioxidant functions. Previously, Chakka et al. (2015) reported that chicken liver protein hydrolysates obtained by fermentation or enzymatic hydrolysis exhibited antioxidant activities in terms of total antioxidant activity, reducing power, and scavenging of superoxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radicals. Thus, both hydrolysates were mentioned to have a good potential to be evaluated as a protein-rich ingredient supporting the reduction of oxidative stress in food products. In a study conducted by Cheng et al. (2016), two types of proteins including blood plasma protein and blood cell protein were isolated from silkie fowl (*Gallus gallus*) blood. Both hydrolysates of these proteins showed strong DPPH radical-scavenging activity and Fe^{2+} chelating ability. The blood plasma protein hydrolysate at 4 h of hydrolysis demonstrated significantly higher antioxidant capacity than those treated by Alcalase in most of the assays. Those hydrolysates were then separated and it was shown that peptides with low molecular weight (<3 kDa) possessed greater antioxidant activity. In another study, peptides were obtained by hydrolysis of chicken skin gelatin with Alcalase, Pronase E and Collagenase enzymes combined with ultrafiltration. The highest antioxidative activity among the three hydrolysate fractions was observed in the lowest molecular weight chicken skin hydrolysate (<2 kDa). Further, the purified chicken skin peptide indicated higher antioxidant activity in terms of hydroxyl radical activity, superoxide anion radical activity and Fe^{2+} chelating activity when compared with the commercial antioxidants (Sarbon et al. 2018). Similarly, hydrolysates obtained from chicken combs and wattles using Alcalase and applying 240 min of hydrolysis showed excellent Fe^{2+} chelating and antioxidant capacities, reducing Fe^{3+} and inhibiting DPPH and ABTS radicals, as reported by Bezerra et al. (2020). The researchers stated that those hydrolysates could be regarded as candidates to be utilized as ingredients to design functional food formulations. In a different study, Nadalian et al. (2019) extracted elastin hydrolysate from poultry skin by using elastase, and all the purified peptide fractions using ultrafiltration, gel filtration chromatography and high-performance liquid chromatography (RP-HPLC) yielded antioxidative activities, where the sequences GAHTGPRKPFKPR, GMPGFDVR and ADASVLPK were identified as antioxidant peptides. The authors emphasized that the presence of hydrophobic amino acids (mainly valine and proline) could potentially associated with the high antioxidant activity of the hydrolysate. In a different research, chicken feather meal was hydrolyzed by using different microbial proteases, and the highest levels of DPPH and ABTS radical scavenging activities were recorded in the hydrolysates derived using 5% Neutrase and digested for 4 h, and low molecular weight-peptides (<0.65 kDa) played an essential part in antioxidant activity (Jeampakdee et al. 2020). Prajapati et al. (2021) also reported high free radical scavenging abilities in feather keratin hydrolysate that were attributed to the amino acid profile having high levels of hydrophobic amino acids such as alanine, cystine, isoleucine, leucine, methionine, phenylalanine, and proline. Ozturk-Kerimoglu et al. (2023) prepared chicken feet protein hydrolysates using combinations of different proteases during different hydrolysis periods and identified peptides exerting antioxidant activity after simulated gastrointestinal digestion. The results indicated that hydrolysis with Alcalase® and Protana® Prime combination for 4 h resulted in the highest antioxidant activity specifically in terms of ferric-reducing antioxidant power (FRAP) and ABTS assays. After peptide identification, the synthesized dipeptides CF and GY showed the highest antioxidant capacity in terms of ABTS radical scavenging activity and oxygen radical absorbance capacity (ORAC). The researchers emphasized that chicken feet protein hydrolysates contain antioxidant peptides that could be evaluated as safe and economical sources to retard oxidation in food systems.

3.2. Antihypertensive activity

The angiotensin-converting enzyme (ACE) hydrolyzes angiotensin-I, resulting in the potent vasoconstrictor angiotensin-II; additionally, ACE inactivates the vasodilator bradykinin. Since ACE activity contributes to increased blood pressure, inhibition of this enzyme is considered a key process to treat hypertension (Callegaro et al. 2018). The use of synthetic ACE inhibitors, such as captopril, lisinopril, and enalapril has been associated with adverse side effects. Consequently, there has been a growing interest in the development of natural antihypertensive compounds and ACE inhibitors as alternatives for reducing blood pressure (Bravo et al. 2019). Therefore, food protein-derived bioactive peptides possess significant potential as natural ACE inhibitors.

De Souza et al. (2017) obtained protein hydrolysates from chicken viscera using different commercial proteases where the hydrolysates were used as a source of bioactive peptides with angiotensin I-converting enzyme (ACE) inhibitory activities (antihypertensive activity). The results showed that enzymatic hydrolysis enhanced the biological properties of the proteins, increasing their ACE inhibitory activities. The maximum values of inhibition detected were 82.1, 82.5 and 83.7% using the binary mixture of Flavourzyme® and Alcalase®, the ternary mixture of Flavourzyme®, Alcalase®, and Neutrase®, and solely Neutrase®, respectively. Callegaro et al. (2018) prepared feather hydrolysates by using three feather-degrading *Bacillus* strains. With increasing feather concentrations, soluble protein was higher with *Bacillus* sp. CL18. In vitro antihypertensive activity of this FH was demonstrated through inhibition of ACE (89.7%), with also antioxidant and antidiabetic potentials, underlining microbial conversion as an eco-friendly technology for the valorization of poultry waste. In another research conducted by Bravo et al. (2019), peptides from chicken foot proteins were separated into fractions by ultrafiltration and RP-HPLC, and subsequent peptide identification in the two fractions that present the highest ACEI activities was carried out. The peptides AVFQHNCQE and QVGPLIGRYCG exhibit antihypertensive activity when administered at an oral dose of 10 mg kg^{-1} body weight, suggesting that those peptides could be used as functional ingredients with antihypertensive effects.

3.3. Antimicrobial activity

Peptides can inhibit the action of strains of pathogenic and altering microorganisms such as gram-positive (*Listeria monocytogenes*, *Bacillus cereus, Staphylococcus aureus, Micrococcus luteus)* and gram-negative (*Escherichia coli*, *Yersinia enterocolitica*) ones. The antimicrobial activity of the peptides is mostly attributed to their low molecular weight as well as α-helical conformation (Borrajo et al. 2019). In a study performed by Chakka et al. (2015), chicken liver protein hydrolysates prepared using fermentation exhibited strong antibacterial activity against *L. monocytogenes* and *B. cereus*, besides moderate inhibition rates for *M. luteus* and *Y. enterocolitica* were also recorded. On the other hand, enzymatically hydrolyzed chicken liver proteins showed antibacterial activity against *M. luteus* but those hydrolysates could not inhibit the other microorganisms. The researchers associated the stronger antibacterial activity of fermented chicken liver hydrolysates with the formation of small peptides by hydrolysis.

3.4. Antidiabetic activity

Diabetes is a glucose metabolic disorder that is considered one of the biggest challenges associated with a complex complication of health crises in the modern lifestyle. Inhibition of dipeptidyl peptidase-IV (DPP-IV) enzyme activity is considered one of the promising therapeutic strategies for the management of type-2 diabetes (Nong & Hsu, 2021). Various antidiabetic biopeptides have been isolated from different food proteins including meat sources. Callegaro et al. (2018) stated that although DPP-IV inhibitory activities were mainly reported for food proteins particularly derived from milk origin, feather hydrolysates produced by *Bacillus* sp. displayed the ability to inhibit DPP-IV. This study suggested that protein hydrolysates of poultry by-products have a good potential for exerting antidiabetic activity.

4. CONCLUSIONS

The poultry meat sector is continuously evolving and expanding. During poultry meat production, a significant amount of by-products with high organic content are generated. The valorization of poultry by-products, particularly those with high potential for bioactive peptides, is crucial for adding value to these products, obtaining economic benefits from waste utilization, and contributing to sustainable environmental practices. Therefore, it is projected that research in this area continue to advance in the future, aiming to elucidate the diverse bioactivity characteristics of by-products and investigate the roles of specific peptide sequences on bioactivity. Furthermore, exploring innovative techniques for the extraction and purification of bioactive peptides from poultry by-products could enhance their commercial viability and broaden their applications in various industries.

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