

# The potential of meat and slaughterhouse by-products as sources of bioactive peptides: A literature review

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**Abstract.** The meat and slaughterhouse industry produces two products, namely prime cut and by-products. By-products in some areas are considered waste and thrown away, but there are also some areas that used as animal feed or edible materials that can be consumed directly by humans. By-products from meat and slaughterhouse industry can be in the form of bone, skin, offal, blood, feather, and feet. Many studies show that by-products from meat and slaughterhouse industry also have high protein content and have potential as source of peptides with certain biological activities. Bioactive peptides are molecules composed of 2-20 amino acids in a certain sequence and have biological activity that is not active in the structure of the parent protein. Some of the biological activities of peptides sourced from livestock by-products are antioxidant, antidiabetic, and ACE inhibitor. Bioactive peptides from livestock by-products have also received much attention from researchers, due to their low cost and abundant sources. Several methods have been developed to produce bioactive peptides, such as chemical, enzymatic, and fermentation methods. The aim of this review was to update and summarize several recent studies regarding bioactive peptides from livestock by-products and their potential biological activities.

## 1 Introduction

The livestock industry plays a vital role in the food sector. The livestock product processing sector comprises several subsectors, including the meat and slaughterhouse industry, dairy industry, and egg industry. Each subsector produces main products and by-products. In general, the term by-product is defined as a part other than carcass, which is edible and non-edible. The meat and slaughterhouse industry produces main products in the form of carcass and by-products in the form of hide/skin, offals, feet, bone, and blood. Some by-products can be utilized as feed and fertilizer, blood being converted into blood meal and used as animal feed has been common. The bone and skin/hide contain collagen which can be used as a raw material for gelatine. The skin/hide is also a valuable raw material in the process of making

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shoes and bags [1]. These by-products are known to have a high protein content. Turkey liver, chicken head, goat skin, and beef lungs are reported to contain protein content of 21.9, 12.29, 31.05, and 19.07%, respectively [2-5]. These data show that the study of protein from livestock by-products is interesting and the potential for the development of bioactive peptides from livestock by-products is also still high.

Proteins, the main components of animal tissues and products, are composed of numerous amino acids linked together by peptide bonds [6]. Proteins in meat muscles can be grouped into 3, namely myofibril proteins, sarcoplasm, and stroma. These groups of proteins have their own characteristics based on their solubility properties in water and salt. Stromal proteins are a group of proteins isolated from connective tissue, so they are also called connective tissue proteins. Some examples of stromal proteins are collagen and elastin. Collagen is a type of protein that is abundant in vertebrates and is the most abundant component in livestock by-products. Collagen is a useful biomaterial in the food, pharmaceutical, and biomedical industries. Collagen is also known to be a good precursor of bioactive peptides [7]. Connective tissue proteins can produce hydrophobic amino acids and bioactive peptides that have antioxidant activity through hydrolysis process. The utilization of by-products into bioactive peptides can increase their economic value and potentially can be applied to the nutraceutical and pharmaceutical industries [8].

Bioactive peptides are fragments of a protein that are known to have certain biological functions, such as antioxidant, antimicrobial, antihypertensive, and mineral binding. The composition and sequence of amino acids can influence the activity of a peptide when it is released from its parental protein. Bioactive peptides generally have a residue length of between 2-20 amino acids [9]. Bioactive peptides are a group of biological molecules that are inactive in their parent protein structure and become active when separated from their parental protein [10]. Bioactive peptide can be produced by chemical hydrolysis using acids and bases and enzymatically using protease enzymes. Chemical hydrolysis using acids and bases causes amino acids to denature due to extreme pH. The use of acids and bases during the hydrolysis process is also considered ineffective, because their action is not specific. On the other hand, the advantage of enzymatic hydrolysis is its specific work. The disadvantage of the enzymatic method is that the price of enzymes is quite expensive. Protease enzymes can come from animal, vegetable and microbial sources. Some examples of protease enzymes are papain, bromelain, pepsin, trypsin, and alcalase [11].

By-product source	Method	Biological activities	Reference
<i>Kacang</i> goat ( <i>Capra hircus</i> ) skin collagen	Enzymatic hydrolysis: thermolysin	ACE inhibitor	[12]
Pork ( <i>Sus scrofa</i> ) and chicken ( <i>Gallus gallus</i> ) skin collagen	Enzymatic hydrolysis: collagenase or a protease from <i>Bacillus licheniformis</i> (MPRO NX®)	Lipase pancreatic inhibitor	[13]
Chicken ( <i>Gallus gallus</i> ) skin	Enzymatic hydrolysis: alcalase and combination pepsin+pancreatin	Antioxidant	[14]
Bovine ( <i>Bos taurus</i> ) and porcine ( <i>Sus scrofa</i> ) collagen	-	Antioxidant and hypoglycemic activity	[15]

Duck ( <i>Anas platyrhynchos</i> ) liver	Enzymatic hydrolysis: alcalase, neutrase, and papain	Antioxidant	[16]
Porcine ( <i>Sus scrofa</i> ) liver	Enzymatic hydrolysis: alcalase, bromelain, papain, and flavourzyme	Antioxidant	[17]
Chicken ( <i>Gallus gallus</i> ) intestine	Enzymatic hydrolysis: fungal protease	$\alpha$ -amylase inhibitory activity	[18]
Turkey ( <i>Meleagris gallopavo</i> ) head collagen	Enzymatic hydrolysis: alcalase, flavourzyme, and trypsin	Bile acid binding and plasma amine oxidase (PAO) inhibitory activity	[19]
Turkey ( <i>Meleagris gallopavo</i> ) trachea	Enzymatic hydrolysis: Alcalase, flavourzyme, protamex, and papain	Antioxidant and ACE inhibitory activity	[20]
Chicken ( <i>Gallus gallus</i> ) bone	Enzymatic hydrolysis: papain, neutral protease, and trypsin	Antioxidant	[21]
Deer ( <i>Cervus elaphus</i> ), sheep (Perendale), and pig (Duroc) blood plasma	Enzymatic hydrolysis: papain, bromelain, fungal protease concentrate 400, and fungal protease II	Antioxidant	[22]

## 2 Materials and Methods

Bioactive peptides can be obtained by several methods, such as chemical, enzymatic, and microbial fermentation methods. Each method has its own peculiarities, disadvantages and advantages. The chemical method is cheap and easy to apply, but some amino acids such as tryptophan can be completely destroyed by acid hydrolysis [23]. In addition to the chemical hydrolysis method, enzymatic hydrolysis and microbial fermentation are more preferred. Both of these methods are usually preferred because they work more specifically, making them suitable for producing bioactive peptides and avoiding amino acid denaturation. In the enzymatic hydrolysis method, the enzymes used come from the proteolytic enzyme group. These proteolytic enzymes can be of animal, vegetable, and microbial origin. Some commonly used enzymes are pepsin, trypsin, alcalase, flavourzyme, papain, and bromelain [11]. Chinese pond turtle protein hydrolysate using alcalase and flavourzyme showed the highest ability to inhibit linoleic acid peroxidation compared to trypsin and bromelain. In the other hand, bromelain hydrolysate exhibited the strongest  $\alpha$ -amylase inhibitory activity compared to other enzymes. Each enzymes have the specificity to produce bioactive peptides that have certain biological activities [24]. The disadvantage of this method is the price of enzymes that tend to be expensive. Meanwhile, microbial fermentation uses microbes that can produce proteolytic enzymes that will cut long chains of proteins into simple peptides. This method has a high diversity of protease enzymes. Microbial fermentation generally uses lactic acid bacteria (LAB). Some researches showed that LAB can produce bioactive peptides

from milk. Based on these three methods above, microbial fermentation method is the most effective method in terms of obtaining bioactive peptide, because microorganism can produce not only one protease, which leads to more bioactive peptide production [11].

Method	Advantage	Disadvantage
Chemical	Cheap and easy to apply	Some amino acids such as tryptophan can be completely destroyed by acid hydrolysis
Enzymatic	Easy to control, specifically producing bioactive peptide, and avoiding amino acids denaturation	Expensive
Microbial fermentation	Producing not only one protease, which leads to more bioactive peptide production	-

### 3 Results and discussion

Various by-products from the meat industry have high protein content that has the potential to produce bioactive peptides with certain biological activities which can be seen in Table 1.

#### 3.1 Skin/hide

Skin/hide in ruminants reaches approximately 3-12% of live weight, while in poultry it can reach 20%. The skin is mostly composed by a major component referred to as collagen. Collagen is a structural protein in the skin that makes up connective tissue, such as bone and skin [25]. Collagen hydrolysate from kacang goat skin using thermolysin showed an ACE inhibitory activity. Peptides with MW <3 kDa showed the strongest ACE inhibitory activity when compared to fractions 3-5 and >5 kDa. Collagen hydrolysate from kacang goat skin at <3 kDa fraction reported to have an ACE inhibitory activity value of 36.27-91.26% in the range of protein concentration 0.60-1.80 mg/ml and the IC<sub>50</sub> value was 82.94 µg/ml [12]. Lipase pancreatic inhibitory activity was reported from pork and chicken skin collagen hydrolysate and peptide fraction. The longer incubation time in the range of 0-24 hours at the time of hydrolysis in chicken and pork skin collagen using collagenase and protease from *Bacillus licheniformis* (MPRO NX®) showed an increase in the degree of hydrolysis and activity of lipase pancreatic inhibitory. The hydrolysate had a similar or even higher ability to inhibit lipase activity than the ultrafiltrated fractions [13]. Meanwhile, antioxidant activity was reported from chicken skin protein hydrolysate using alcalase and combination pepsin-alcalase. This study have shown that protein hydrolysate from chicken skin and its fractions had the antioxidant activity. Chicken skin protein hydrolysate can scavenge DPPH, hydroxyl, peroxy, superoxide radicals, and also chelate ferrous ion. Protein hydrolysates can act as antioxidants through the mechanism of electron donation to free radicals [14]. The ability to chelate iron ions is also often associated with the presence of histidine residues that have imidazole rings [26]. Bovine and porcine collagen hydrolysate exhibited antioxidant and hypoglycemic activity through in vitro test. However, α-amylase and α-glucosidase inhibitory activity from bovine and porcine collagen hydrolysate was lower than the acarbose

as positive control [15]. The inhibitory activity of  $\alpha$ -amylase enzymes by bioactive peptides can come from amino acid residues of Tyr, Trp, Phe, Lys, and cationic residues that tend to bind  $\alpha$ -amylase [24].

### 3.2 Liver

Liver contains high protein, which is around 20%. The liver can also be a good source of several vitamins, such as vitamins B2, B3, B6, B12, ascorbic acid, and vitamin A [16-17, 27]. Duck liver protein hydrolysate by alcalase, neutrase, and papain enzymes showed antioxidant activity. The duck liver protein hydrolysate using the alcalase enzyme had the highest ABTS and hydroxyl scavenging activity, when compared with other enzymes. Six antioxidant peptide sequences (GEHGDSSVPVWSGVN, HLDYYLGK, HLPWIGK, DTYIRQPW, WDDMEKIWHH, and MYPGIAD) were successfully isolated and identified from duck liver protein hydrolysate using alcalase. These six peptides were also known to increase SOD and CAT activities and decrease MDA production and ROS in H<sub>2</sub>O<sub>2</sub>-induced HepG2 [16]. Protein hydrolysate from porcine liver has also been reported to have potential as an antioxidant. Several peptide sequences that can be candidates as antioxidants were APAAIGPYSQAVLVDR, GLNQALVDLHALGSAR, ALFQDVQKPSQDEW GK, LSGPQAGLGEYLFER, and LGEHNIDVLEGNEQFINAAK [17].

### 3.3 Intestine

Chicken intestine protein hydrolysate showed  $\alpha$ -amylase inhibitory activity. The IC<sub>50</sub> of the chicken intestine protein hydrolysate, chicken intestine peptide fractions <10 kDa, <3 kDa, and acarbose were 273.04, 959, 362.86, and 158.42 mg/ml, respectively. The highest  $\alpha$ -amylase inhibitory activity was in acarbose, however the chicken intestine protein hydrolysate and its fractions still had potential as an antidiabetic [18].

### 3.4 Head

The protein content of turkey heads was 9.67%, which showed a lower protein content compared to chicken heads, which was 12.29%. Collagen from turkey head was hydrolyzed using single enzyme (alcalase, flavourzyme, and trypsin), dual enzyme (alcalase-flavourzyme, alcalase-trypsin, and flavourzyme-trypsin), and cocktail of all 3 enzymes together. The collagen peptides from all treatments exhibited bile acid binding capacity and PAO inhibitory activity. The increasing of collagen peptide concentration from turkey head increased the percentage of cholic and deoxycholic acids. This shows that the peptide can be an antihypercholesterolemic agent through the mechanism of binding bile acids, thereby preventing reabsorption of bile acids and reducing the amount of cholesterol in the blood. Turkey collagen peptides also inhibited PAO. The ability to inhibit PAO can be used to evaluate the ability of a peptide to act as an anti-inflammatory [3 and 19].

### 3.5 Trachea

Chicken trachea is a by-product from slaughterhouse. The protein content of chicken trachea was 69.71% on a dry basis. Chicken trachea flakes was reported to have a good solubility at the pH range 6-10 and isoelectric point at pH 4. The soluble protein content in chicken trachea hydrolysate was increasing during 0-6 hours hydrolysis time using papain and alcalase, however the soluble protein content was decreasing after 2-3 hours hydrolysis time in flavourzyme and protamex treatment. Antioxidant and ACE inhibitory activity were

reported in chicken trachea hydrolysate. The treatment using alcalase showed the highest antioxidant activity through DPPH and ABTS assay. The lowest antioxidant activity was showed by papain treatment, while protamex and flavourzyme treatment showed the intermediate antioxidant capacity between papain and alcalase. There was a volatility in antioxidant activity among all the treatments during the hydrolysis time. Peptides have the potential to donate hydrogen atoms that can scavenge free radicals. Chicken trachea hydrolysate also exhibited ACE inhibitory activity. The IC<sub>50</sub> ACE inhibitor was reported at 0.42, 0.73, 0.55, and 0.72 mg/ml for alcalase, flavourzyme, protamex, and papain, respectively. The greatest ACE inhibitory activity was showed by alcalase. IC<sub>50</sub> means the concentration needed to make 50% inhibition. The most abundant amino acids in all chicken trachea hydrolysate were glutamine, glycine, alanine, and proline. Ala and Pro were reported to have antihypertensive activity [20 and 28]. Several ACE inhibitory peptides were reported from chicken parent protein (myosin, creatine kinase, actin, and collagen). Four ACE inhibitory peptides were reported from the hydrolysate of boneless chicken leg meat. The sequences peptide were MNVKHWPWMK, INDNFYDWLP, WDWPY, and VTVNPKWLP, two peptides (P1 and P4) among the sequences were from myosin [28].

### 3.6 Bone

Chicken bone protein hydrolysate, its fractions, and their Maillard reaction products showed ability to scavenge DPPH and hydroxyl radical and showed reducing power. Increasing of concentration in all treatments can increase the antioxidant activity. Maillard reaction products derived from peptide fractions had stronger antioxidant activity (DPPH radical scavenging activity and reducing power) than the hydrolysate and its peptides. The Maillard reaction is often occurred between carbonyl and amino groups in food processing. Antioxidant activity from peptides can be increased through the Maillard reaction with reducing sugar. Maillard reaction product derived from protein hydrolysate-sugar has potential for development [21].

### 3.7 Blood plasma

Blood represents approximately 4% of animal weight live. It's rich in protein and iron. Blood can be used in feed industry in the form of blood meal for feeding livestock and pet [1]. Blood plasma protein hydrolysate from deer, sheep, and pig using papain, bromelain, FP400, and FPII exhibited antioxidant activity, but didn't exhibit antimicrobial activity. Enzymatic hydrolysis can change the structure of native protein and open and exposes amino acid residues that can react with oxidants [22]. Porcine blood plasma hydrolysate produced using alcalase also exhibited antioxidant activity through TBARS, DPPH, reducing power, and metal chelating activity test. Increasing of degree of hydrolysis (DH) increased reducing power, DPPH scavenging, and copper and ferrous ion chelating activity dan decreased TBARS value. The highest reducing power and DPPH radical scavenging activity was obtained in fraction <3 kDa compared to fractions 3-6, 6-10, and >10 kDa. The peptide sequence from porcine plasma protein with the highest antioxidant activity was identified as His-Asn-Gly-Asn [29].

## 4 Conclusion

By-products from meat and slaughterhouse industry is rich in protein. This also shows that by-products from the meat industry and slaughterhouses have the potential to produce

bioactive peptides. Production of bioactive peptides from by-products can increase their economic value and support the development of the functional food and nutraceutical industries. Several studies have shown that peptides derived from by-products of the meat industry and slaughterhouses have several biological activities, such as antioxidant,  $\alpha$ -amylase inhibitory activity, ACE inhibitory activity, lipase pancreatic inhibitory activity, PAO inhibitory activity, and bile acids binding capacity.

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