Research article

Bioactive peptides from meat industry by-products as potential antimicrobial agents based on BIOPEP-UWM database

Paulina Kęska*, Joanna Stadnik, Anna Kononiuk, Justyna Libera, Karolina M. Wójciak

Department of Animal Raw Materials Technology, Faculty of Food Science and Biotechnology, University of Life Sciences in Lublin

*paulina.keska@up.lublin.pl

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Abstract: *New peptides with potential antimicrobial activity, encrypted in protein sequences of meat industry by-products were searched with bioinformatics tools using BIOPEP-UWM database. The potential of major proteins as a source of biologically active peptides with antibacterial, antiviral and antifungal activity were considered. As a result, collagen, hemoglobin, fibrinogen and selected meat tissue proteins (creatine kinase, myosin, titin) has been shown to contain short motifs responsible for antibacterial properties. The peptides with antiviral and antifungal properties were not detected.*

Keywords: *antibacterial, antiviral, antifungal, peptides, meat industry, in silico.*

Introduction

Natural antimicrobial peptides (AMPs) have been identified and characterized in virtually all living organisms, from prokaryotes to humans. These peptides have been recognized as ancient evolutionary molecules that have been effectively preserved in mammals. They are produced as natural mechanisms of innate immunity in organisms against invasive pathogens. Antimicrobial peptides have different mechanisms of action [1] and exhibit antibiotic activity with a broad spectrum of action against microorganisms, including bacteria, fungi and viruses. Their protein origin directs the search for other, innovative sources of AMPs, such as protein-rich food [2]. Generally, food-derived proteins are rich source of bioactive compounds, which can be classified as antihypertensive, antioxidant, immunomodulatory, opoid, anticoagulant, anti-obesity and mineral-binding, depending on biological functions. Their antimicrobial activity has also been noticed, especially when they are produced using bacterial proteases, for example during fermentation process. These bioactive peptides can also be easily produced by enzymatic hydrolysis and during gastrointestinal digestion.

Antimicrobial peptides made from food proteins are a great advantage that they can be obtained from natural and safe sources, which is why they can be expected to be safe when used as supplements or drugs as well as by the food industry. Therefore, food proteins can be considered not only because of their nutritional value, but also as a potential antibacterial, antiviral or antifungal agent, acting as an ingredient supporting food-borne immunity. In the food safety context, antimicrobial peptides derived from industrial waste can be used as preservatives for the storage and distribution of food products.

As pointed by Mirabella et al. [3], the frequency of meat consumption is increasing worldwide, while the demand for less valuable products such as blood, guts and some muscles that have been commonly consumed in the past because of the need for poverty is on the decline. For this reason, the meat industry rejects large amounts of slaughter by-products, which mainly cover the meat scraps, limbs, skin, bones, viscera, fat tissues, feet, skulls and other. By-products from meat waste constitute almost 60-70% of the slaughtered carcass, of which almost 40% form edible and 20% inedible [4]. They can present a number of environmental and economic problems because of the need for their utilization [5]. Toldrá et al. [6] analyzed the potential uses of meat wastes, distinguishing three recovery methods: human food, pet food and other non-food and non-feed applications. Many different uses of meat by-products were pointed, not only for human and animal food, but also for biotechnology and the chemical industry. Furthermore, the use of these by-products for extracting proteins and peptides is more preferable than rejecting them as waste. It is claimed that biotechnological methods, such as enzymatic hydrolysis and fermentation, are better than chemical ones. Thus, waste materials containing proteins is becoming more and more attractive for the production of bioactive peptides. In recent years, there has been growing global pressure on the food industry to try and minimize the environmental impact of its activities and to improve sustainability. This has led to increased interest in the more complete recovery and optimum utilization of by-products from the food industry [7].

Although a number of studies have been conducted so far focusing on the production of bioactive peptides from a variety of food sources, there are only limited studies investigating the production of bioactive peptides from meat industry by-products. Observation that antimicrobial peptides can be generated from parent proteins that usually have a different physiological function in the body has prompted us to look for their atypical sources. Their properties could be used against microbial food contamination or as a factor supporting human immune systems.

The aim of this study was to evaluate the potential of the selected protein sequences from meat industry by-products as a precursor of antimicrobial, antiviral or antifungal peptides based on *in silico* methods.

Experimental

Materials

Selected meat industry by-products were analyzed by the *in silico* approach towards their use as antimicrobial agents. The pork (*Sus scrofa*), beef (*Bos taurus*) and lamb (*Ovis aries*) raw materials were chosen as the most often consumed. The meat (as scraps), collagen (bone component), blood cells (fibrinogen, myoglobin, hemoglobin) as waste in meat processing were used. All proteins and they types were selected according to literature data and availability of sequences in databases [8]. The meat tissues are characterized by high protein content, about 19% in the muscle tissue, depending on the species. Generally, they are divided into three main groups of proteins. The first of them, the most abundant (50-55%) are myofibrillar proteins such as myosin, actin, troponin complex, tropomyosin, α-actinin, titin, nebulin. They are involved in muscle contraction mechanisms (contraction proteins, regulators, cytoskeleton and Z lines). The second fraction are the sarcoplasmic proteins, representing 30-35% of the total protein content, which acts as enzymes and chromoproteins. This includes, but is not limited to, dehydrogenase, phosphorylase, enolase or kinase. The smallest fraction are connective tissue proteins (1.5-10%), which strengthen the tissue structure. The sarcoplasmic and myofibrillar proteins were selected as the predominant group of meat proteins from meat tissue scraps of slaughter animals. Collagen was selected as the main protein from trimming bones. Type of collagen and number of chain was chosen on the basis of literature data, concerning information about chain composition and tissue distribution of different types of collagen [9, 10]. Therefore collagen type I, II, III and V were chosen as the classical fibril collagens which occurs mainly in bones, cartilages and other trimming bones. Blood is generated in very large volumes as a by-product in slaughterhouses. In blood there are three main type of proteins: fibrinogen, serum globulins and albumin. Separated fractions are use in food and dietary supplements [11]. Types of blood proteins to analysis were selected as the most abundant blood's components. Hemoglobin is globin, accounting for more than half of the proteins present [12]. Hemoglobin and myoglobin are used in many meat products as a colouring factor of meat products. Subunits alpha and beta were chosen as the two basic subunits of hemoglobin. Subunits theta, zeta and epsilon were chosen as an alpha or beta-type chain of embryonic hemoglobin. Fibrinogen occurs as a dimer, where each monomer is composed of three non-identical chains, which were analyzed (alpha, beta and gamma), linked together by several disulphide bonds [11, 12]. All sequences were obtained from the UniProtKB database (Swiss-Prot; TrEMBL)[8] and were presented in Table 1.

Methods

The potential of the selected protein sequences as precursor antimicrobial motifs were evaluated based on BIOPEP-UWM database [14, 15] The profile of their potential biological activity, defined as the type, number and location of

bioactive fragments in a protein chain were detected. The value of selected proteins as bioactive peptide precursors were evaluated based on the occurrence frequency of the fragments with a given activity in a polypeptide chain (*A*) defined as: $A = a/N$; where a is the number of fragments with a given activity in the protein chain and N is the number of amino acid residues in the polypeptides chain of a protein molecule.

Table 1. Various types of meat by-product proteins used in *in silico* study

Waste material	Protein	*Bos taurus*	*Ovis aries*	*Sus scrofa*
Trimmings bones	Collagen type I, alpha 2 chain	P02465	W5NTT7	Q1T7B0*
	Collagen type I, alpha1 chain	P02453	W5P481	A0A287A1S6
	Collagen type II, alpha1 chain	P02459	W5QDH3	I3LSV6
	Collagen type III, alpha 1 chain	P04258	W5Q4S0	*n.a.***
	Collagen type V, alpha 1 chain	G3MZI7	W5NVR8	Q8HYS4*
	Collagen type V, alpha 3 chain	*n.a.*	W5Q4M3	Q8HYS2*
Blood	Hemoglobin subunit alpha	P01966	P68240	P01965
	Hemoglobin subunit beta	P02070	P02075	P02067
	Hemoglobin subunit theta	*n.a.*	W5PN58	P04246
	Hemoglobin subunit zeta	*n.a.*	W5PMJ4	P02009
	Hemoglobin subunit epsilon	P06642	A0A0F6YEG8	P02101
	Myoglobin	P02192	P02190	P02189
	Fibrinogen alpha chain	P02672	W5Q5H8	Q28936*
	Fibrinogen beta chain	P02676	W5NQ45	F1RX37
	Fibrinogen gamma chain	P12799	W5Q5A6	Q6R6M8*
	Fibrinogen C domain	E1BDM4	W5PBX3	F1S0X3
	Serum albumin	P02769	W5PWE9	P08835AY9
At scraps	Creatine kinase U-type	Q9TTK8	*n.a.*	Q29577
	Creatine kinase M-type	Q9XSC6	W5PJ69	Q5XLD3
	Glyceraldehyde-3-phosphate dehydrogenase	W5PDG3	Q2KJE5	P00355
	L-lactate dehydrogenase	W5NXL6	B0JYN3	P00339
	Phosphoglycerate kinase 1	B7TJ13	Q3T0P6	Q7SIB7
	Alpha-1,4 glucan phosphorylase	W5PB77	B0JYK6	F1RQQ8
	Fructose-bisphosphate aldolase	A6QLL8	W5P1X9	Q6UV40
	Pyruvate kinase	Q3ZC87	W5P275	A0A287B8G0
	Actin, alpha skeletal muscle	P68138	W5NYJ1	P68137
	Myosin-2	Q9BE41	W5PT09	Q9TV63
	Tropomyosin alpha-3 chain	Q5KR47	W5NUU3	A1XQV4
	Troponin C, skeletal muscle	Q148C2	A8WEG2	P02587
	Troponin T, fast skeletalmuscle	Q8MKI3	W5NRC71	Q75NG9
	Troponin T, slow skeletal muscle	Q8MKH6	W5NUR7	Q75ZZ6
	Titin	F1N757	W5Q754	Q29117*
	Nebulin	F1MQI3	W5PFV9	Q3Y5G4

*fragment; **sequence not available

Results and discussion

In addition to existing synthetic compounds, there is a need to investigate new antimicrobial candidates for combating pathogenic factors that reduce the quality of food products as well as those that cause disease after consumption. From this point of view, food proteins are a source of this biologically active ingredients that help in the better functioning of the human body. Researchers are looking for new peptide-based compounds against unwanted microflora from alternative sources, e.g. from various food sources or waste products. Antiviral peptides (AVPs) are a potential alternative strategy in this context. AVP have been experimentally tested to disrupt the key developmental stages of pathogenic human viruses. Research on the antiviral properties of milk proteins was carried out by Floris et al. [16]. Particular attention has been directed to the antiviral activity against human immunodeficiency virus (HIV) and the human cytomegalovirus (HCMV). The authors point out that unmodified milk proteins are usually not active against these viruses. The exception is lactoferrin, which has significant antiviral activity against both HIV and HCMV. Several other milk proteins tested showed strong antiviral activity only after chemical modification (by making them polyanionic - for anti-HIV or polycationic activity - for anti-HCMV activity). Thus, the AVPs from food or waste sources could then be used as a starting point for the design of more active molecules targeted at combating viral infection. For this purpose, the use of *in silico* methods can be helpful [17].

Fungi, including molds are another form of food contamination or poisoning. Admittedly, certain species are desirable in traditional food production recipes, such as dry ripening sausages where a mold contribution is required to achieve their particular taste. However, the toxinogenic molds which can also appear, are the most dangerous. To control unwanted mold forms, physical and chemical methods are not suitable, especially for meat products [18]. Untypical sources, rich antifungal proteins and peptides are quite a promising remedy for combating unwanted molds in food, thus reducing food poisoning. Nevertheless, no peptide sequences with antifungal or antiviral properties from meat industry by-products in this study was detected.

Table 2. Antimicrobial potential (*A parameter*) of selected proteins from *Bos taurus, Ovis aries* and *Sus scrofa*

Protein	*Bos taurus*	*Ovis aries*	*Sus scrofa*
Collagen type I, alpha 2 chain	0.0007	0.0007	*not detected*
Hemoglobin subunit alpha	0.0141	0.0070	0.0071
Hemoglobin subunit zeta	*n.a**	*not detected*	0.0071
Fibrinogen alpha chain	0.0016	*not detected*	*not detected*
Fibrinogen gamma chain	0.0045	0.0046	0.0106
Fibrinogen C domain	0.0022	*not detected*	0.0022
Creatine kinase U-type	0.0024	*n.a*	0.0024
Creatine kinase M-type	0.0052	0.0052	0.0052
Myosin-2	0.0010	*n.a.*	0.0010
Titin	0.0004	0.0003	0.0017

* sequence not available

The interest on antibacterial peptides have been observed, mainly due to their ability to quickly kill microorganism cells, rapid spectrum of action, low cytotoxicity and resistance to gastrointestinal enzymes. Such peptides are synthesized on ribosome for plants and animals and may be subject to post-translational modifications. Antibacterial peptides may be also generated by proteolytic protein degradation. Nevertheless, studies are limited mainly to milk proteins and egg white [19, 20]. Antibacterial peptides were also found in *in silico* approach in various meat origin by-products according to Table 2. Of these, collagen, hemoglobin, fibrinogen and selected meat tissue proteins contained short sequences responsible for these specific potential antibacterial properties. From trimmings bones, only one sequences recognize as tetrapeptides CIRA obtained from Collagen type I, alpha 2 chain were detected. At the past, CIRA as antibacterial peptide of lactoferrin from cheese whey was detected by other researches [21]. Importantly, this antimicrobial peptide did not occur in *Sus scrofa*, which is probably due to the genetic conditioning of differences in collagen sequences. It should be noted that *Bos taurus* and *Ovis aries* belong to the *Bovidae* family, while *Sus scrofa* should be classified as a mammal of the *Suidae* family.

Raw animal blood is essentially divided into two by-products after centrifugation: plasma (the colorless part) and cruor (the red fraction). The plasma is a good source of proteins, like thrombin, fibrinogen, immunoglobulin G or serum-albumin [22]. About cruor, it is the fraction which gives blood its red color, representing 45% of the whole blood, and it contains mainly hemoglobin [12, 23]. According to the results, hemoglobin subunit alpha contains sequences responsible for action against bacteria, especially when of bovine origin (A = 0.0121) (importantly – 142 amino acids in peptide sequence for *Bos taurus* and *Ovis aries* and 141 amino acids for *Sus scrofa*). Also zeta subunit of porcine hemoglobin was the source of the bioactive peptides (A = 0.0071). So far, several antimicrobial peptides produced from animal proteins, including hemoglobin, were described in the literature. For example, the VTLASHLPSDFTPAVHASLDKFLANVSTVL peptide from bovine hemoglobin exhibited antibacterial activity against *Micrococcus luteus* A270, *Listeriainocua, Enterococcus faecalis, Bacillus cereus, Staphylococcus saprophyticus* and *Staphylococcus simulans* [24]. It should be noted that the best known antibacterial peptides derived from *Sus scrofa* and *Bos taurus* are long motifs, from 12 to 79 amino acids in the sequence [25]. Most peptides with antimicrobial activity are present in cationic form. Lopez-Exposito et al. [19] noted that short peptides, *e.i.* YVL and IQY are peptides that do not have positively charged residues. It is likely that these short motifs do not follow the general mechanism of action described for longer cationic antibacterial peptides. In the present study, two peptides from various hemoglobin subunits were identified, i.e. the pentapeptide TSKYR and the tripeptide YVL (Table 3), thus relative shorter sequences, which are more likely to be achieved, for example, during hydrolysis. The obtained result is consistent with reports by Przybylski et al. [23], who

generated and isolated the TSKYR peptide from bovine hemoglobin, through enzymatic hydrolyze of bovine cruor, a slaughterhouse co-product. The TSKYR peptide was used for meat as a food preservative in the preliminary tests and the authors of this study showed that TSKYR inhibited microbial growth during refrigeration storage by 14 days and reduced lipid oxidation in meat by about 60% retarding meat rancidity.

Table 3. Antibacterial peptide sequences identified in selected proteins [peptide localization placed in parentheses]

Protein	*Bos taurus*	*Ovis Aries*	*Sus scrofa*
Collagen type I, alpha 2 chain	**CIRA** [1241-1244]	**CIRA** [1241-1244]	*not detected*
Hemoglobin subunit alpha	**TSKYR** [142-146]	**TSKYR** [142-146]	**TSKYR** [141-145]
Hemoglobin subunit zeta	*n.a*.*	*not detected*	**YVL** [91-93]
Fibrinogen alpha chain	**IQY** [227-229]	*not detected*	*not detected*
Fibrinogen gamma chain	**IQY** [238-240] **YVL** [277-279]	**IQY** [239-241] **YVL** [276-278]	**YVL** [27-29]
Fibrinogen C domain	**YVL** [30-32]	*not detected*	**YVL** [30-32]
Creatine kinase U-type	**YVL** [162-164]	*n.a.*	**YVL** [162-164]
Creatine kinase M-type	**YVL** [129-131], [287-289]	**YVL** [129-131], [287-289]	**YVL** [129-131], [287-289]
Myosin-2	**IQY** [199-201], [845-847]	**IQY** [199-201], [842-844]	**IQY** [199-201], [844-846]
Titin	**IQY** [24907-24909], [30057-30059] **YVL** [15805-15807], [15969-15971], [18281-18283], [22163-22165], [24503-24505], [25869-25871], [27694-27696], [31328-31330], [31631-31633], [31775-31777], [33461-33463]	**IQY** [25797-25799], [30947-30949] **YVL** [16695-16697], [16857-16859], [19171-19173], [23051-23053], [25391-25393], [28584-28586], [32216-32218], [32519-32521], [32663-32665], [34352-34354]	**IQY** [390-392]

* sequence not available

Fibrinogen is another factor associated with blood as a production waste. It is a key player in the blood coagulation system, and is upon activation with

thrombin converted into fibrin that subsequently forms a fibrin clot. Påhlman et al. [26], studied the role of fibrinogen in the early innate immune response in the human body. The authors have shown that the GHR28 peptide fragment released from the fibrinogen β-chain has antimicrobial activity against bacteria. In particular, bacterial killing was detected in Group A *Streptococcus* bacteria trapped in fibrin clot, suggesting that fibrinogen and coagulation are involved in the early innate immune system to quickly block and neutralize invasive pathogens. According to these reports, also globules of animal origin could show satisfactory antibacterial effects, in particular fibrinogen C domain of *Bos taurus* and *Sus scrofa* (A = 0.0022 in both cases; sequence length 460 and 461 respectively). Fibrinogen alpha (only from *Bos taurus*) and fibrinogen gamma (in particular protein from *Sus scrofa*) proved to be their most abundant source (A = 0.106) also containing antibacterial sequences, such as IQY, YVL. Admittedly, they were previously identified in different milk fractions, which is not surprising, since the *in silico* approach allows the search for new, alternative peptide sources with previously described sequences with confirmatory properties. Moreover, one of the strongest antibacterial peptide compounds, lactoferricin, is derived from bovine milk protein, i.e. lactoferrin obtained by pepsin digestion [27]. According to other reports, the synthetic peptides, i.e. YVL reduced the initial count of *S. marcescens*, and the peptide IQY showed a bactericidal effect against *E. coli*. In addition, both peptides showed strong activity against Gram-positive bacteria [17]. Mentioned amino acids sequences were also recognized in meat scraps, especially creatine kinase (both type), myosin-2 and titin molecules. The role of myosin (in *in silico* approach) and titin (in *in vitro* approach) is emphasized in the literature as a potential source of antimicrobial sequences [25, 28]. However, according to our knowledge, there has been no previous information about creatine kinase from meat of slaughter animals as a source of antibacterial peptides. Castellano et al. [28] identified a highly active antilisterial peptide among the naturally generated peptides in Spanish dry-cured ham. Moreover, peptide sequences were identified from the most active fractions against eight *Listeria* strains after RP-HPLC separation, showing from short peptide sequences (5 to 18 amino acids in length), which is in accordance with observations presented in this study. This results indicate that meat scraps also can be a source of peptides against bacteria when they are released from proteins by the hydrolytic cleavage of the peptide bond, as it happens during Spanish ham ageing.

Conclusion

The antibacterial activity of bioactive peptides, such as CIRA, TSKYR, IQY, YVL from by-products can play an important role in preventing food spoilage or treating diseases related to foodborne poisoning associated with bacteria thus giving innovative value-addition to such meat by-products. These bioactivities together with their safety profile and the costs associated with the removal of meat by-products makes bioactive meat-derived peptides an attractive option for

use as natural compounds that improve the safety of the product or ingredients in functional food products. However, meat by-products are currently under-utilized and limited by cultural, religious and traditional factors. For some cultures, the transformation of by-products through peptide manufacturing processes may be unacceptable to consume. In addition, so far not available economically economical purification and standardization of bioactive meat-derived peptides limits development and production on a large scale. Nevertheless, the trend of using by-products is likely to be strongly considered, yielding value-added products and significantly reducing waste generation. To limit these difficulties, the *in silico* approach is a cheap and efficient alternative. However, it should keep in mind the fact that *in silico* methods obtained results should be verified using experimental methods.

References

1. Bahar A, Ren D. Antimicrobial peptides. Pharmaceuticals **2013**, 6:1543-1575.
2. Biziulevicius GA, Kislukhina OV, Kazlauskaite J, Zukaite V. Food-protein enzymatic hydrolysates possess both antimicrobial and immunostimulatory activities: a "cause and effect" theory of bifunctionality. FEMS Immunol Med Microbiol **2006**, 46:131-138.
3. Mirabella N, Castellani V, Sala S. Current options for the valorization of food manufacturing waste: a review. J Clean Prod **2014**, 65:28-41.
4. Bhaskar N, Modi VK, Govindaraju K, Radha C, Lalitha RG. Utilization of meat industry by products: protein hydrolysate from sheep visceral mass. BioresourTechnol **2007**, 98:388-394.
5. Lafarga T, Hayes M. Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. Meat Sci **2014**, 98: 227-239.
6. Toldrá F, Aristoy MC, Mora L, Reig M. Innovations in value-addition of edible meat by-products. Meat Sci **2012**, 92:290-296.
7. Otles S, Despoudi S, Bucatariu C, Kartal C. Food waste management, valorization, and sustainability in the food industry. In Food Waste Recovery Galanakis CM ed. Galanakis Laboratories, Chania, Greece **2015**, 3-23.
8. UniProtKB database; http://www.uniprot.org/ (access: November 2017).
9. Engel J, Bächinger HP Structure, stability and folding of the collagen triple helix. In Collagen: primer in structure, processing and assembly. Brinckmann J, Notbohm H, Müller PK Eds., Springer Berlin Heidelberg **2005**, 7-33.
10. Ricard-Blum S, Ruggiero F, van der Rest M The collagen superfamily. In Collagen: primer in structure, processing and assembly. Brinckmann J, Notbohm H, Müller PK Eds., Springer Berlin Heidelberg **2005**, 35-84.
11. Lynch SA, Mullen AM, O'Neill EE, García CÁ. Harnessing the potential of blood proteins as functional ingredients: A review of the state of the art in blood processing. Comp Rev in Food Sci F 2017, 16:330-344.
12. Hsieh YH, Ofori JA. Food-grade proteins from animal by-products. Their usage and detection methods. In Handbook of analysis of edible animal by-products. Nollet LML, Toldrá F Eds., CRC Press Boca Raton FL, USA **2011**, 3-11.
13. Ofori JA, Yun-Hwa PH The use of blood and derived products as food additives. In Food Additive. El-Samragy Y. Ed., InTech Rijeka Croatia **2012**, 229-256.

14. BIOPEP-UWM; http://uwm.edu.pl/biochemia/index.php/pl/biopep (Access: November 2017).
15. Minkiewicz P, Dziuba J, Iwaniak A, Dziuba M, Darewicz M. BIOPEP database and other programs for processing bioactive peptide sequences. J AOAC Int **2008**, 91:965-980.
16. Floris R, Recio I, Berkhout B, Visser S. Antibacterial and antiviral effects of milk proteins and derivatives thereof. Curr Pharm Des **2003**, 9:1257-1275.
17. Thakur N, Qureshi A, Kumar M. AVPpred: collection and prediction of highly effective antiviral peptides. Nucleic Acids Res **2012**, 40:199-204
18. Delgado J, Peromingo B, Núñez F, Asensio MA. Use of molds and their antifungal proteins for biocontrol of toxigenic molds on dry-ripened cheese and meats. Curr Opin Food Sci **2016**, 11:40-45.
19. Lopez-Exposito I, Minervini F, Amigo L, Recio I. Identification of antibacterial peptides from bovine κ-casein. J Food Prot **2006**, 69:2992-2997.
20. Mine Y, Ma F, Lauriau S. Antimicrobial peptides released by enzymatic hydrolysis of hen egg white lysozyme. J Agric Food Chem **2004**, 52:1088-1094.
21. Recio I, Visser S. Two ion-exchange chromatographic methods for the isolation of antibacterial peptides from lactoferrin: *In situ* enzymatic hydrolysis on an ion-exchange membrane. J Chromatogr A **1999**, 831:191-201.
22. Bah CS, Bekhit AEDA, Carne A, McConnell MA. Slaughterhouse blood: an emerging source of bioactive compounds. Compr Rev Food Sci Food Saf **2013**, 12:314-331.
23. Przybylski R, Firdaous L, Châtaigné G, Dhulster P, Nedjar N. Production of an antimicrobial peptide derived from slaughterhouse by-product and its potential application on meat as preservative. Food Chem **2016**, 211:306-313.
24. Daoud, R, Dubois V, Bors-Dodita L, Nedjar-Arroume N, Krier F, Chihib NE, Guillochon D. New antibacterial peptide derived from bovine hemoglobin. Peptides **2005**, 26:713-719.
25. Kęska P, Stadnik J. Antimicrobial peptides of meat origin-an *in silico* and *in vitro* analysis. Protein Peptide Lett **2017**, 24: 165-173.
26. Påhlman LI, Mörgelin M, Kasetty G, Olin AI, Schmidtchen A, Herwald H. Antimicrobial activity of fibrinogen and fibrinogen-derived peptides–a novel link between coagulation and innate immunity. Thromb Haemost **2013**, 110: 930-939.
27. Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M. Identification of the bactericidal domain of lactoferrin. Biochim Biophys Acta **1992**, 1121:130-136.
28. Castellano P, Mora L, Escudero E, Vignolo G, Aznar R, Toldrá F. Antilisterial peptides from Spanish dry-cured hams: Purification and identification. Food Microbiol **2016**, 59:133-141.