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WASTE AND BY-PRODUCTS FROM THE MEAT INDUSTRY AS A SOURCE OF BIOACTIVE COMPOUNDS

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Abstract

The aim of this review was to summarize existing knowledge on bioactive peptides from the waste and by-products from meat industry and identify future directions of research. Reducing food waste and transforming it into food and feed is a step toward the achievement of economic development while reducing adverse effects on the environment. Meat production and consumption have increased in recent years. Above 20% of meat is wasted in Europe throughout the different steps in the production chain. Meat waste and by-products are rich sources of proteins, bioactive compounds, essential amino acids, vitamins and minerals, and have a potential to be further used for dietary and non-dietary purposes. Bioactive peptides often have health-promoting effects such as antioxidant, anti-hypertensive, anti-inflammatory, antimicrobial and antitumor activities when ingested and absorbed by human beings. Some bioactive peptides were shown to reduce the risk of development of cancer, diabetes and cardiovascular disease, which are among the most common diseases currently. Several methods to isolate bioactive peptides from meat waste and by-products were developed. Enzymatic hydrolysis can generate hydrolyzates and certain bioactive peptides from larger proteins, and the hydrolyzates can be used as dietary supplements or as an additive to increase the protein content of food. Bioactive peptides can be isolated from the hydrolyzates and used as functional food components, dietary supplements or medicines. We concluded that wastes and by-products from the meat industry present an opportunity for the generation of health-promoting bioactive compounds which can be successfully used in food and other relevant industries. However, existing research mainly is mainly focused on the

influence of individual bioactive peptides on certain health parameters and is mainly performed using *in vitro* methods. Thus, future research should be directed to assess the long-term effects of bioactive compounds as a component of the whole diet.

Key words: animal protein; bioactive peptides; by-products; food security; food waste; meat.

Introduction

An extensive transformation of the existing food production chain and food consumption is vital for reaching the Sustainable Development Goals (SDGs) [1]. The livestock sector is responsible for approximately 14.5% of global greenhouse gas (GHG) emissions and GHG from animal-based foods are twice higher than GHG of plant-based foods [2, 3]. Thus, beef production is responsible for 35.3%, dairy cattle 30.1%, swine 9.5% and poultry 8.7% [4].

Food waste, unwanted and/or unused material of the primary production and/or consumption, is an important issue globally. In 2019, 931 million tons of edible foods were wasted [4]. Reducing food waste reduces the green gas emission, protects natural resources and increases global food security. The demand for food is constantly increasing because of increasing of human population and, consequently, food consumption. Along the production line of food, from field to fork food waste occurs in all stages in all sectors. Reducing food waste in the meat industry is important for both economic and environmental reasons. It was estimated that 23% of the production in the meat sector in Europe 2018 through all stages, from primary production through post-harvest, manufacturing, distribution

and consumption, is wasted [4]. From this, 64% of the waste occurs at the consumption stage.

A number of studies have emerged studying the quantities and types of meat waste generated during the production and consumption stages [5-7]. In those studies, different aspects were considered including reasons for food waste generation, strategies of prevention, and consumer's attitudes and behavior.

Most meat waste includes trimmings, cuttings, bones, collagen, carcasses, skins, fatty tissues, hoofs, internal organs, and blood. The definition of a by-product often depends on traditions, culture and religion; therefore, waste products and by-products definitions vary between geographical regions. Trimmings are meat portions which are left behind after the preparation of primal cuts from the carcass. Trimmings include fat, gristles, and meat, and are obtained by removal of muscle traces from the bones after the deboning process. Head meat, internal organs, major tendons and ligaments are not regarded as trimmings. Up to 30% of the live weight of livestock animals can be considered as edible by-products.

Generally, waste from meat industry is rich in proteins and fat and contain a range of essential minerals

and vitamins [8]. Protein-containing meat waste is an attractive material for the production of bioactive peptides with health-promoting properties [9]. Thus, meat waste generated can be potentially treated as a raw material for production of various biomaterials including food additives, medical preparations and feed material.

Materials and methods

A literature study was conducted using the databases Scopus, Web of Science and PubMed. Peer-reviewed scientific publications have been selected using a combination of several keywords including: meat waste, meat by-products, animal bioactive peptides, meat bioactive compounds, by-product nutritional composition. Relevant networking websites, rapports, authority scientific opinions and conference proceedings have also been evaluated. Only publications written in English were included in the study.

Results and discussion

Categories of meat by-products

Food safety is a critical aspect as unsafe food poses global health threats. Even though by-products might be important sources of nutrients, their safety should be considered. To achieve safe products there are strict rules for the use of meat by-products.

Within the European Union, meat by-products are generally grouped into 3 categories based on their risk to human or animal health [10]. The categories are presented in Table 1. Category 1 includes by-products which have a very high risk, category 2 by-products have from medium to high risk and category 3 by-products have the lowest risk. Animal by-products posing a high risk should only be used for purposes outside the feed chain.

Waste content

Generally, waste from meat industry is rich in proteins and fat and contain a range of essential minerals

Therefore, the aim of the present study was to provide a summary of existing information on bioactive compounds from the waste and by-products from meat industry, identify future challenges and highlight areas where more research is needed.

and vitamins. Protein-containing meat waste is an attractive material to produce bioactive peptides with health-promoting properties [9]. Thus, meat waste and by-products can be potentially used to enhance the nutritional quality and functional value of foods. Edible fats separated during meat processing can be used in bakeries and confectionery, for cooking and frying, and for enhancing the flavor and texture of some foods [11]. Waste products can be used as a source of emulsifying and texturizing agents, colourants and the source of bioactive compounds [12].

Protein in waste and by-products from meat industry

Many meat by-products and waste are rich in proteins and amino acids. Blood is an example of a protein-rich waste product. The amount of blood generated during meat production is high and blood acts as a pollutant for

the environment if disposed directly into water bodies [13]. However, the high protein content of blood makes it potentially useful in the food industry, as it can be used to enhance the nutritional value of other foods. The modern slaughterhouses in developed countries are equipped with waste management lines to separate blood from other parts and collect blood for

animal feed or fertilizer purposes [14]. If the bovine blood has no infectivity risk like TSE, it may be used for pet foods and feeds for livestock [10]. If blood is hygienically collected and managed at approved slaughterhouses, it can be considered for human consumption [11].

Table 1- Meat by-products categories according to the European Union.

Category	Risk	Examples of by-product	Examples of use
1	Very high	Material at a TSE risk, such as Specified Risk Material (e.g. bovine spinal cord). Pet animals, zoo and circus animals.	Destroyed by incineration, or by rendering followed by incineration (TSE suspects). Some material can be pressure-rendered and disposed of in an authorised landfill site. Some material to be used as a fuel for combustion, although there are no existing rules for this. Some material can also be used for the manufacture of medical devices.
2	Intermediate	Fallen stock, manure and digestive content, milk, colostrum	Some material can also be used for the manufacture of medical devices. Production of fuel, biodiesel, biogas. Some material can be pressure-rendered and used for the production of organic fertilisers or in an approved composting or anaerobic digestion plant.
3	Low	Carcasses and parts of animals slaughtered which are not finally destined to human consumption but fit	Production of pet food and organic fertilisers or soil improvers. Production of animal feedingstuffs, though TSE related restrictions on the feeding of processed animal protein restrict this.

		for human consumption. Hides, hair, feathers, bones	
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Skin, bones and cartilages are also rich in proteins, such as collagen. Collagen is widely used in the food, pharmaceutical and cosmetic industries. In the food industry, collagen is used as a food additive and as a packaging material [15]. It is possible to extract collagen from the skin, ears and other waste material from meat industry, as it was demonstrated by many research groups. Collagen from rabbit by-products was also recently extracted and characterized [16]). This is important knowledge for Ukrainian industry as rabbit production will likely increase after the end of Russian military aggression [17].

Thus, efforts are needed to develop novel processes to efficiently

use these waste and by-products and turn them into high-value material.

Bioactive peptides: definition and activity

Bioactive peptides are molecules of 3-20 amino acid residues with beneficial effects on health because of their potential biological activities. Those peptides are inactive when kept within the parent protein, and become active only after the cleavage of the proteins. The health-promoting activities include antimicrobial, antihypertensive, antioxidant, immunomodulatory and anti-inflammatory [18] (Figure 1). To perform their bioactivity, peptides must be absorbed, have low or no toxicity, and do not have an unpleasant taste. Bioactive peptides are easily excreted from the body and do not accumulate.

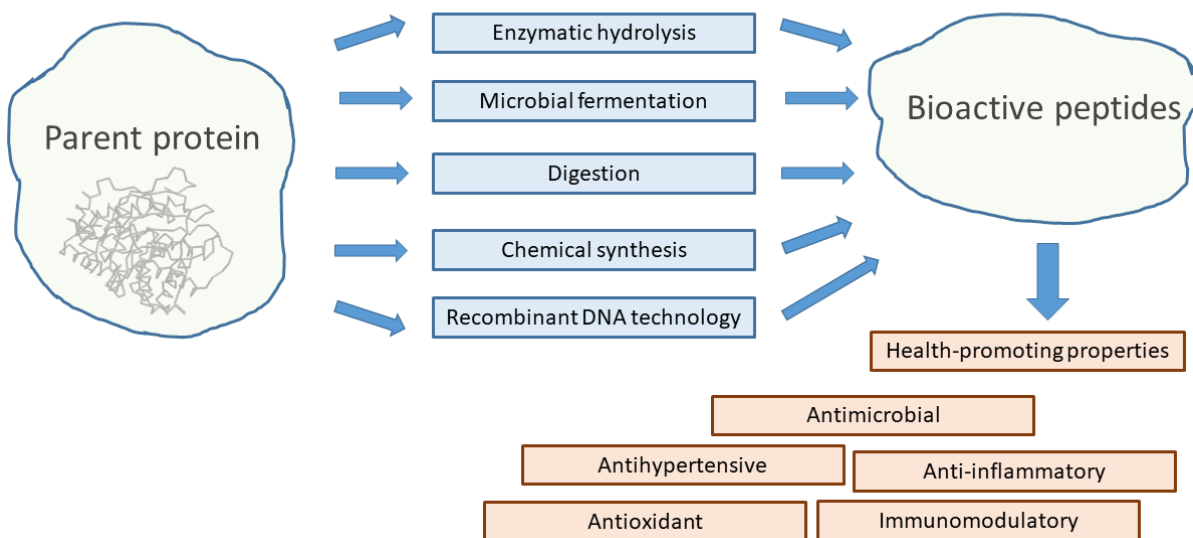


Figure 1 -Production methods and health-promoting properties of bioactive peptides

Generation of bioactive peptides from waste and by-products

Bioactive peptides can be isolated from their original protein through enzymatic hydrolysis and microbial fermentation (Figure 1). If the amino acid sequence of bioactive peptides is known, they can be produced by chemical synthesis or recombinant DNA technology [19] (Figure 1). Bioactive peptides can be isolated from various sources, including dairy and plant-based products, animal-based meat, and waste/by-products from the food industry. There are many types of bioactive peptides which can be produced using various methods. Some bioactive peptides are formed by endogenous enzymes in post-mortem meat, while other peptides - through microbial fermentation and chemical or enzymatic hydrolysis. The proteolytic enzymes originate from animals, microorganisms or plants and the type of proteolytic enzymes and substrate will determine the types of bioactive peptides generated. The most common way to produce bioactive peptides is through enzymatic hydrolysis, because of low amount of used toxic

compounds, high specificity and mild conditions [20]. Another commonly used method to obtain bioactive peptides are microbial fermentation and *in vitro* digestion. In this study, the focus will be placed on enzymatic hydrolysis with proteases.

Enzymatic hydrolysis

During this process, the proteins are hydrolysed, and the bioactive compounds are released. Many proteolytic enzymes can be used, including commercially available pepsin, trypsin, chymotrypsin, corolase PP, papain, bromelain and pronase [11]. Those enzymes need different optimal conditions to perform their action [21]. Enzymatic hydrolysis of bioactive peptides using two types of enzymes, either enzymes extracted from microorganisms or plants, or digestive enzymes [22]. A combination of both types of enzymes can also be used, depending on the structure of the desired peptide.

The success of enzymatic hydrolysis is affected by pH, temperature, enzyme/substrate ratio, length of hydrolysis and enzyme deactivation (Table 2).

Table 2 - Some common commercial enzymes in generation of bioactive peptides, optimal conditions in the process and examples of use

Enzyme name	Optimum temperature, °C	Optimum pH	Origin	Meat by-products for protein hydrolysis	Reference
Papain	60-70	6-7	Papaya fruit	Animal muscle from bovine, porcine or deer	[18]
Bromelain	35-45	7	Pineapple fruit	Deer, sheep and pig blood	

				Porcine liver	
Ficain (ficin)	60	8	Fig tree	Camel meat, beef, and pork	[23]
Pronase	40-60	7.5	<i>Strepto myces griseus</i>	Bovine skin	[24]

A complete procedure includes peptide generation, isolation, characterization and bioactivity assays. The first step (generation of bioactive peptides) is basically a characterization of the meat by-products, which might be potentially used as a protein source. A *in silico* analysis is useful in characterization of the primary, secondary, tertiary and quaternary structure of the proteins. Then, the enzymatic digestion should be performed, where appropriate enzymes are used to generate the desired bioactive peptide [25]. The enzymes suitable for the digestion are determined by the *in-silico* analysis [26]. The hydrolyzates are generated and purified by different separation techniques, for example, ultrafiltration. Then, the hydrolysates are tested for their bioactivities. The hydrolyzates with desired properties are further purified by the use of chromatographic techniques such as ion chromatography (IEC), gel filtration chromatography (GFC) and high-performance liquid chromatography (HPLC). These methods separate the hydrolysate to peptidic fractions, which are further tested for their bioactivity using bioactivity assays. The bioactive peptide fractions with desired properties are selected and

characterized using mass spectrometry techniques such as MALDI-TOF and ESI-MS [27]. The sequenced bioactive peptides are then synthesised for additional bioavailability studies. The synthesis of the bioactive peptides is usually done by liquid-phase synthesis, Fmoc Solid-phase synthesis and Boc Solid-phase synthesis. Lastly, dose-response, safety and bioavailability studies *in vivo* are performed before the bioactive peptide are allowed to be used in the food industry [8].

Bioactive peptides prepared by this method with use of food-grade enzymes are recognized as safe. However, the disadvantages of this method is high cost, low number of commercially available enzymes and a limited peptide yield. Moreover, enzymatic hydrolysis is a time-consuming process and requires control over the temperature, pH, substrate and enzyme concentrations.

Fermentation

Fermentation is another method, which releases potential bioactive peptides by both natural and controlled fermentation. In the generation of bioactive peptides from dairy and plant foods, lactic acid bacteria (LAB) is often used. However, the activity of LAB is not efficient in meat products, and to the best of our knowledge, only limited data is available on bioactive

peptides generated from meat [28]. Even lower number of studies were focused on the generation of bioactive peptides from meat by-products.

Health effects of bioactive peptides

The specific activity of the bioactive peptide is determined by its amino acid sequence, structure, chemical properties, and spatial structure of the peptide chain. Examples of health effects include but not limited to anti-hypertensive, antimicrobial, antioxidant, anti-inflammatory and antitumor activities [20].

Because of the increasing prevalence of hypertension and increased risk of the development of cardiovascular diseases, a lot of researcher are searching for approaches to prevent it by dietary meant. Reduction of blood pressure is important factor in prevention of hypertension. Renin-angiotensin system is responsible for regulation of the blood pressure. Angiotensin is converted to angiotensin I, then angiotensin I is converted to angiotensin II by angiotensin I converting enzyme (ACE). The generation of angiotensin II causes vasoconstriction. Inhibition of ACE leads to the limited formation of angiotensin II and thus lowers vasoconstriction [29]. Saiga et al. [30] demonstrated antihypertensive activity of chicken muscle extract after treatment with *Aspergillus* protease. The GFPTTKTYFPHF and VVYPWT peptide sequences have been shown to have anti-hypertensive activity. The GFPTTKTYFPHF peptide were found

in the α -chain between fragment 34-46, VVYPWT peptide - between the fragments 34-39 on the β -chain of porcine hemoglobin. These peptides act as ACE inhibitors and exert their health effects by reduction of the blood pressure. Many of the anti-hypertensive bioactive peptides act as competitive inhibitors of the ACE enzyme. GFPTTKTYFPHF have an IC₅₀ value of 4.9 μ M and VVYPWT have an IC₅₀ value of 6.0 μ m from porcine blood. IC₅₀ is a value indicating the concentration of inhibitor needed to inhibit 50% of a biological reaction, in this case, the ACE enzyme. ACE-inhibitory peptides corresponding to the sequences of porcine hemoglobin have also been identified [31]. ACE-inhibitory peptides (EACF and CDF) from rabbit meat proteins were also shown to have strong inhibitory effects [32]. In this in vitro study, EACF acted as a competitive ACE inhibitor with IC₅₀ value of 41.1 μ M, and CDF - as a non-competitive inhibitor with IC₅₀ value of 192 μ M [32]. The bovine fibrinogen-enriched protein fraction was also identified as a source of bioactive peptides with ACE-inhibitory activity [33].

Antimicrobial peptides reduce the growth of microorganisms without side effects. The restricted use antibiotics have made antimicrobial peptides an attractive option against pathogens. Antimicrobial peptides from beef sarcoplasmic protein with activity against *Pseudomonas aeruginosa* were identified [34]. The peptide GLSDGEQ showed inhibitory effects against gram-negative and gram-positive bacteria, *Salmonella typhimurium*, *Bacillus*

cereus, *Escherichia coli* and *Listeria monocytogenes* [20]. Hydrolysates from porcine blood proteins also demonstrated antimicrobial effect against *Bacillus cereus* [35].

Bioactive peptides with antioxidant activity might inhibit the effect of free radicals and reactive oxygen species. Antioxidants neutralize free radicals by donating electrons and stabilizes the free radicals making them less reactive, thus inhibiting the free radical ability to react with other substances in the human body [36]. The fact that some antibiotics cause side effects and oxidative damage, led to the search of new compounds with antioxidant properties. In this regard, bioactive peptides are an attractive option because of no side effects.

Bioactive peptides also continue to attract attention in clinical tumor therapy. Some bioactive peptides can act directly or indirectly on tumor cells and change the growth and apoptosis of the tumor cells. The antitumoral bioactive peptides generally act by inhibiting tumor angiogenesis and enhancing tumor cell apoptosis [37]. Currently, several peptides with antitumoral activities were isolated

Conclusion

Some meat by-products are suitable for human consumption and can be consumed either directly or after processing. Some of meat by-products can be transformed into protein-rich feed for pets and livestock animals. Meat by-products and meat waste which are rich in proteins and produced in large amounts, can be used for the generation of bioactive peptides. Although there is a trend in the

from bovine meat [34], and many studies indicated the potency of meat waste and by-products as a source of antitumor peptides [12].

Functional food with added bioactive peptides

Bioactive peptides from waste and by-products with health-promoting properties are promising ingredients for functional foods. Surprisingly, research on product development and effects of food with added bioactive peptides is limited. Wheat bread prepared with an enzymatic hydrolysate of bovine α - and β -globulins and fed to spontaneously hypertensive rats, led to a reduction of systolic blood pressure after 2 h of administration, although after 24 h, blood pressure increased [38]. When pork blood and liver hydrolysates were included as an ingredient in pork loaves, physico-chemical (water activity, lipid oxidations, color, texture and microbial qualities) and sensory properties were acceptable [39]. More studies are needed on improved processing, sensory evaluation and health effects of using bioactive peptides from meat industry as an ingredient in functional foods.

European Union to limit the consumption of animal-based proteins, meat by-products and waste have the potential to be used in the production of bioactive peptides and enhance food properties. Bioactive peptides can exert health-promoting properties and are considered as compounds for the development of functional foods. They might exert anti-hypertensive, antimicrobial, antioxidant, anti-

inflammatory and antitumor activities. Bioactive peptides are released from parent proteins by several methods, among which enzymatic hydrolysis and fermentation are the most common. Enzymatic hydrolysis is performed using single or combination of enzyme to release the desired peptides. Some of these enzymes are derived from plants or microorganisms, some are animal digestive enzymes (trypsin and pepsin).

Because of increased demands for large-scale production of bioactive

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peptides from meat by-products and waste, more research is needed to identify their activity and to develop efficient, cheap and reliable methods for their generation. Moreover, future research should be directed to assess short- and long-term effects of bioactive compounds as a component of the whole diet and investigate possible effects of interactions with other food ingredient.

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