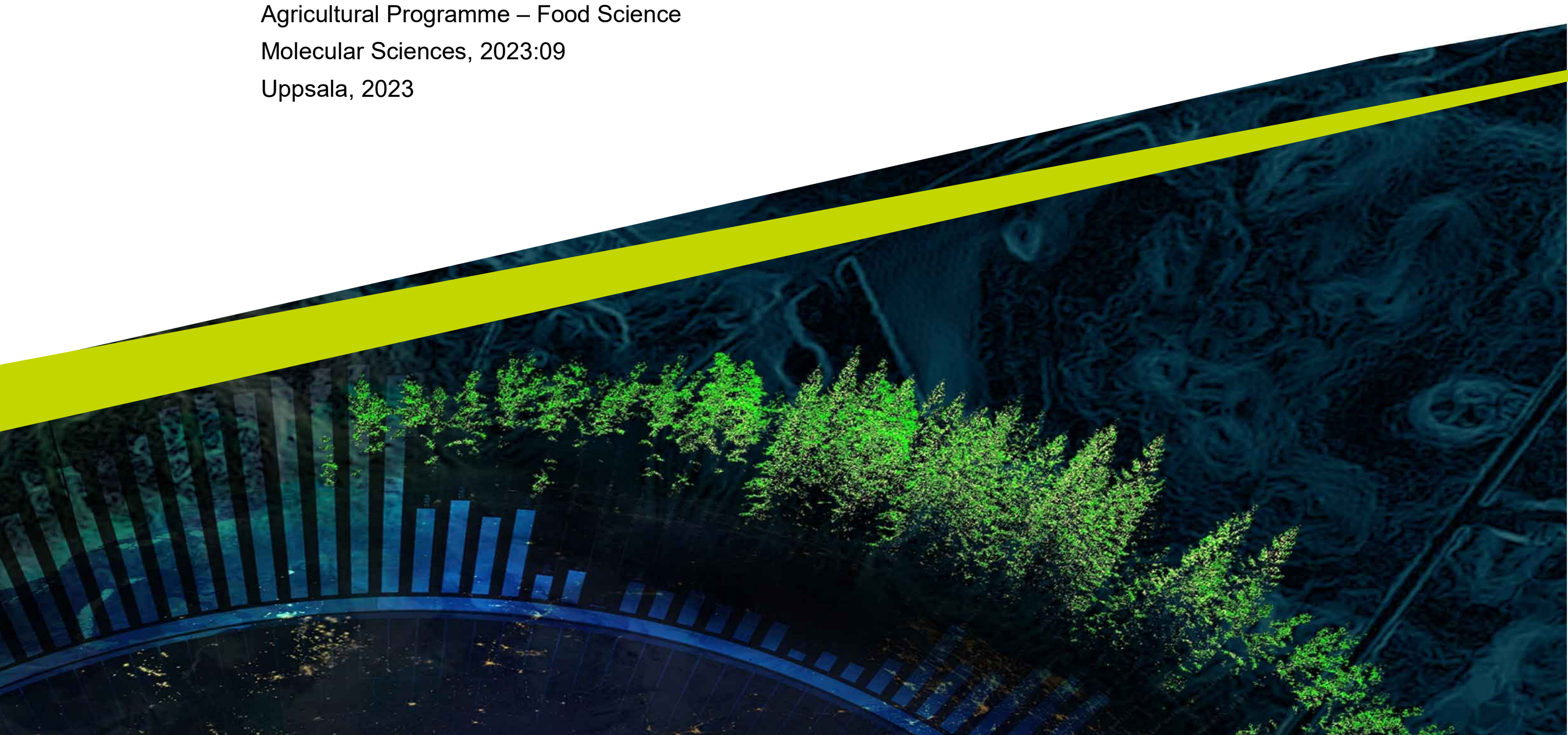
Sustainable utilization of waste and by-products from the meat industry

Anton Glimaker

Independent project in food science • Bachelor´s thesis • 15 credits
Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences, SLU
Agricultural Programme – Food Science
Molecular Sciences, 2023:09
Uppsala, 2023



Sustainable utilization of by-products from the meat industry

Hållbart utnyttjande av avfall och biprodukter från köttindustrin

Author's name Anton Glimaker

Supervisor:	**Galia Zamaratskaia, SLU, Department of molecular science**
Examiner:	Saeid Karkehabadi, SLU, Department of Molecular Science

Credits:	15 credits
Level:	First cycle, G2E
Course title:	Independent project in Food Science
Course code:	G2E
Programme/education:	Agricultural Programme – Food Science
Course coordinating dept:	Department of Molecular Sciences
Place of publication:	Uppsala
Year of publication:	2023

Title of series:	Molecular Sciences
Part number:	2023:09
Keywords:	Food waste, sustainability, protein, bioactive peptides, enzymes

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences (NJ)
Department of Molecular Sciences

Abstract

The purpose of this research was to summarise existing scientific literature on waste and by-products from the meat industry and suggest how the meat industry can use it in order to reduce environmental damage, increase food availability and contribute to the achievement of the Sustainable Development Goals. The report is a literature study were several databases were used.

In 2018, 340 millions tons of meat were produced and 23% were wasted in Europe throughout the different steps along the production line of food, which includes from field to fork. 64% of all waste occurred at the consumption stage, 19% - at the manufacturing stage, 11% - in primary production and post-harvest and 5% - at the distribution stage. There are many reasons that waste occur and the reason differs depending on the stage.

Meat production and consumption have increased in recent years. A reduction of meat waste in the meat industry leads to economical growth and better environment because it reduces the release of greenhouse gases such as CO_2, methane and meat by-products with high pollutant characteristics.

Meat waste and by-products include trimmings, cuttings, bones, collagen, carcasses, skins, fatty tissues, hoofs, internal organs and blood. All of these are rich source of proteins, bioactive compounds, essential amino acids, vitamins and minerals. Therefore they have a potential to be further used.

Bioactive peptides often have health-promoting effects such as antioxidant, anti-hypertensive, anti-inflammatory, antimicrobial and antitumor activities when ingested or absorbed. Bioactive peptides can help to prevent many diseases such as cancer, diabetes and cardiovascular disease which are among the most common diseases at the moment.

Waste and by-products can be used in many ways. Some of them are already being used, for example, trimmings in hotdog production, but others are not. Enzymatic hydrolysis can be used to generate hydrolyzates and certain bioactive peptides from larger proteins, and the hydrolyzates can be used as dietary supplements or can be incorporated 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. However, more research is needed.

Keywords: waste, sustainability, protein, bioactive peptides, enzymes

Table of contents

List of tables

List of figures

Abbreviations

ACE	Angiotensin-converting enzyme
AME	Adsorptive-mediated endocytosis
EC number	The Enzyme Commission number
PepT1	Peptide transport 1
SDGs	Sustainable Development Goals

1. Introduction

1.1 Background

Sustainability is an important long-term goal for modern society. Reducing food waste, and transform food waste and by-products into medicines, feed and food is a big step to meet the needs of economic development while reducing adverse impact on the environment.

Figure 1. The global development goals adopted 25 september 2015 as part of the 2030 agenda (Wikipedia 2022). https://commons.wikimedia.org/wiki/File:Sustainable_Development_Goals.jpg [2023-05-16].

It contributes to several Sustainable Development Goals (SDGs): goal 1 (no poverty), 2 (zero hunger), 3 (good health and well-being), 8 (decent work and economic growth), 9 (industry, innovation and infrastructure), 11 (sustainable cities and communities), 12 (responsible consumption and production), 13 (climate action) and 15 (life on land) (Figure 1).

Therefore actions are required to maximize the utilization of the produced food. Since the adoption of the SDGs in 2015, a lot of research have been focused on reduction of food waste and sustainable use of by-products. This research has added great theoretical knowledge, but implementation of this knowledge in the food chain is still limited.

1.2 Aim

The aim of this study was to summarise existing scientific literature on waste and by-products from the meat industry and suggest how meat industry can use this in order to reduce environmental damage, increase food availability and contribute to the achievement of SDGs.

2. Methods

Scientific information was collected through a literature study using the databases Sciencedirect and PubMed. Peer-reviewed scientific publications have been selected using combination of several keywords such as: meat waste, meat by-products, meat bioactive peptides, by-product nutritional composition, and peptide absorption. Moreover, two interviews were contacted with researchers on waste and by-products from meat industry, Professor Anders Karlsson, Swedish University of Agricultural Sciences (SLU), Katarina Arvidsson Segerkvist, researcher at SLU for the department of environment, health of pets and production systems and Associate Professor Kadyrzhan Makangali from S.Seifullin Kazakh Agro Technical University, Kazakhstan and Galia Zamaratskaia university lecturer at the department of molecular sciences. Both interviews were conducted via zoom and the questions were prepared in advance.

3. What is waste and why we need to reduce it

3.1 Definition of waste and benefits of waste reduction

Food waste is defined as unwanted and/or unused material that remains after the primary production and/or consumption. Food waste is a significant issue in the world. In 2019, 931 million tons of edible foods were wasted. Reducing food waste provides various benefits for individuals and planet. It reduces the green gas emission, increases global food security, protects natural resources, ecosystems and increases access to food for people in need. Malnutrition is an increasing global problem. The demand for food is on a steady rise due to the increase in population and consumption of food. Along the production line of food, from field to fork food waste occurs in most stages. Donation of edible parts of food to social services which otherwise would have been wasted are one of the best solutions to reduce food waste. In 2012 the percentage wasted of all food through the different stages of the food chain was 65% at the consumption stage, 19% in the manufacturing stage, 11% in primary production and post-harvest and 5% at the distribution stage (Karwowska et al. 2021). Meat production in the world increases despite the negative impacts on the release of green gas emissions. In 2018, meat production was 340 million tons, while fifty years ago meat production was around 113 million. There has been an overall increase in meat consumption over the years. Reducing food waste in the meat sector is important for both economic and environmental reasons. Food animal production around the world contributes to 14.5% of the human-induced greenhouse gas emissions per year, this is equal to 7.1 gigatons of CO_2. Beef production is responsible for 35.3%, dairy cattle 30.1%, swine 9.5% and poultry 8.7% (Karwowska et al. 2021). It was estimated that 23% of the production in the meat sector in Europe 2018 through all stages, which include primary production and post-harvest, manufacturing, distribution and consumption, is wasted. 64% of the waste happens in the consumption stage, 20% in the manufacturing stage, 12% in the distribution stage and 3.5% in the primary production and post-harvest stage. In North Africa, West and Central Asia, Latin America, South and Southeast Asia, Sub-saharan Africa, most of the loss and waste happens in the production and storage levels. Meat has the highest emissions per kilogram of food compared to other foods (Karwowska et al. 2021). Thus, there are several reasons to reduce waste from meat production; it reduces the release of greenhouse gases such as methane from cows and increases the financial security of producers and consumers. Reduction of the amount of waste will also indirectly contribute to improved global food security and nutrition.

3.2 Major reasons for food loss at the different stages of the supply chain

There are many reasons for food loss which depend on the stage of the supply chain.

Food loss in primary production is due to farming/rearing conditions and the storage conditions during transport. For example, 0.1% of pigs are killed during transport and 0.1% are rejected during slaughter (Cederberg & Sonesson 2011). Animal sickness is another reason for loss of livestock, especially if the diseases can spread quickly through livestock. Meat from sick animals is removed from the supply chain, to eradicate disease and protect consumers.

Losses at the processing step account for 20% of all the losses of the supply chain. A few reasons of the food losses during the processing stage include incorrect transport, product changes, human error and product defects (Karwowska et al. 2021). Spoilage is a common reason for food loss because meat products are easily perishable. Meat products needs to be stored at a low temperature, a common reason for spoilage is an unexpected increase in the temperature. The shelf life of meat products is relatively short. The main reason for wastage of meat products in the retail store is if meat products do not get sold before its labelled expiry date. Inadequate meat quality during the processing may result in losses due to the presence of pathogenic microorganisms. Meyer et al. (2010) identified Campylobacter and Salmonella as the most frequent cause of bacterial enteritis in Germany. Improper packaging also causes loss of meat products, this occurs during storage and transport of products. Poor or incorrect organization of food distribution might lead to damage to packaging material and waste.

The consumption stage in the food supply chain is responsible for the largest amount of waste. The consumption stage includes households, catering, and restaurants. Meat food waste in households, catering and other food services stands for 64% of the total amount of waste of all stages in the supply chain (Karwowska et al. 2021). In general, households generate more waste than the food services. One of the reasons for the waste at the consumption stage is that individuals both at food services and households have a low awareness about the sustainability-oriented innovation opportunities and the challenges that arise if we do not minimize food waste. A significant amount of the food which is bought by restaurants does not reach the customer and gets wasted. About 31-40% of meals served are not consumed (Karwowska et al. 2021). Consumer's attitude towards food waste play a major role in how much food people waste. To inform people about how to prevent and manage food waste is a good strategy in reducing waste because this can improve people's attitudes regarding food waste and recycling. Other common reason for food waste at the consumption stage is misunderstanding of the label, improper storage, left-over and consumer plate waste, consumer confusion about best-beforere dates, preparation of too much food, inadequate staff competence, the purchase of low-quality products, psychological tastes, attitudes and preferences leading to plate waste (I don´t eat that) and inadequate meal planning. For consumers, improper cold storage, failure of freezing and insufficient knowledge about how to prepare the food are common reasons for meat waste. Some studies have shown that change of plate size and type of plate might reduce the waste (Ofei et al. 2015). Adjustments of school's nutrition plan can reduce food waste as well as information campaigns about food waste (Karwowska et al. 2021).

3.3 Waste content

Most meat waste inlcude trimmings, cuttings, bones, collagen, carcasses, skins, fatty tissues, hoofs, internal organs, and blood. The definition of a by-product is influenced by 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.

Generally, waste from meat industry is rich in proteins and fat and contain a range of essential minerals and vitamins (Kowalski et al. 2021). Protein-containing meat waste is an attractive material for the production of bioactive peptides with health-promoting properties (Ryder et al. 2016). 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.

4. Importance of proteins, amino acids and bioactive peptides for human health

4.1 Proteins and amino acids

Proteins are essential macronutrients and together with carbohydrates and fats provide energy to support basic functioning and physical activity. The main role of the proteins is to build the cells and tissues and they play a major role in the regulation of physiological processes.

Proteins are polymers of amino acids. Amino acids are important building blocks in the body. The human body uses amino acids to build and repair bones, muscles, and tissue, making enzymes and hormones from amino acids. Enzymes and hormones are critical for human health, as they regulate not only physiological functions but also mood and well-being. Protein consists of a total of 20 amino acids, 9 of which are essential, which the body cannot produce and therefore they have to be taken through food. The essential amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

A lot of meat waste and meat by-products are rich in proteins and amino acids. Blood is an example of a protein-rich waste product. The amount of generated blood is high and blood is characterised as pollutant for the environment (Al-Gheethi et al. 2021). However, high protein content in blood makes it useful in the food industry, and can potentially be used to enhance the nutritional value of foods, and act as water-binding and emulsifying agent. Skin, bones and cartilages are also rich in proteins, such as collagen (Peydayesh et al., 2023). Thus, efforts are needed to develop novel processes to efficiently use these waste and by-products and turn them into high-value material.

4.2 Bioactive peptides

Meat waste is as a good source for bioactive peptides (Mora et al. 2014). Bioactive peptides are short sequences of amino acids ranging from 2 to 30 amino acids. Many bioactive peptides have health promoting effects in the human body when ingested. These effects include antioxidant activity, anti-hypertensive activity, anti-inflammatory activity, antimicrobial activity, and antitumor activity (Mora et al. 2014). It is the amino acid sequence of the bioactive peptide which determines its health promoting effects. The best sources to obtain bioactive peptides from animals include blood, trimmings, bones, internal organs and skin**.** Examples of bioactive peptides originated from bones are QYDQGV, YEDCTDCGN and AADNANELFPPN. These peptides comes from buffalo horns (Lafarga & Hayes 2014).

Bioactive peptides from blood mainly originates from hemoglobin and plasma proteins, these bioactive peptides have been described to have antihypertensive, antioxidant, antimicrobial and opioid activity (Mora et al. 2014).

Collagen is also a good source of bioactive peptides. Most studies have indicated that bioactive peptides from collagen have angiotensin-converting enzyme (ACE)-inhibitory and antioxidant activity (Mora et al. 2014).

4.2.1 Absorption of peptides by the human body

The peptides are absorbed by the intestine system. Peptides are released to the circulation and interact with different cells, tissues and organs to express its bioactivity. For the bioactive peptide to exert its activity in the body the original peptide sequence needs to be intact (Lafarga & Hayes 2014).

Intestinal absorption of small peptides was recently reviewed by Shen and Matsui (2019). Briefly, bioactive peptides can be hydrolysed in the gastrointestinal tract by different enzymes. The absorption of bioactive peptides occurs in the brush border of the lumen in the small intestine. The bioactive peptides can be absorbed through the paracellular route, peptide transport 1 (PepT1) mediated route, transcytosis route and passive transcellular diffusion through or beside the enterocyte in the small intestine and then released into to the blood circulation. These absorption routes are poorly understood and the exact mechanisms of absorption are uncertain. The body prefers the absorption of single amino acids, di-peptides and tri-peptides over long chain peptides, because smaller peptides are easier to be transported. There are intracellular and brush border enzymes such as peptidases which include aminopeptidase, tri-peptidase and imino di-peptidase that can hydrolyse peptides into amino acids, di- and tri-peptides (Xing et al. 2021).

Di-peptides and tri-peptides are absorbed intact by the peptide-specific transport system. Small peptides get absorbed by the peptide transporter T1 which is present in the enterocyte in the brush border of the small intestine. Peptides larger than 3 amino acids are not absorbed by PepT1 (Sai et al. 1998). Amino acids are absorbed via sodium-dependent cotransport.

Intestinal adsorptive-mediated endocytosis (AME) is an absorption route that can absorb peptides larger than 3 amino acids (Sai et al. 1998). The knowledge about absorption of such peptides was mainly obtained by *in vitro* studies and have to be treated with caution. *In vitro* studies are not enough to be able to determine a certain peptides bioavailability, because the *in vitro* studies cannot exactly mimic the conditions inside the human body and can only give indications of the bioavailability of the bioactive peptide (Xing et al. 2021). Further in *vivo* studies are needed to assess bioavailability of meat derived bioactive peptides. At the moment most medicinal peptides which are used clinically are administered through the paranteral route and not orally because of peptides poor bioavailability. However modification of peptides such as site-specific drug delivery, chemical modification, use of vehicles and adjuvants, bioadhesive drug delivery, penetration enhancers, protease inhibitors, nano and microparticulate carriers and multifunctional polymeric excipients for example. These new modification tools can raise the oral bioavailability of peptides and make them an attractive option in clinical medicine (Patel & Misra 2011).

5. How waste and by-products can be used

Waste and by-products can be used to enhance nutritional and functional value of foods. Waste products can be used as emulsifying and texturizing agents, colourants and the source of bioactive compounds (Mora et al. 2014).

5.1 Generation of bioactive peptides from waste and by-products

Bioactive peptides can be generated and isolated through enzymatic hydrolysis, cooking and fermentation from their original protein. Bioactive peptides can be generated from various sourses, including milk, fish, egg, soy, meat, waste and by-products from food industry. There are many types of bioactive peptides which can be produced using various methods. Some bioactive peptides can be generated with help of endogenous enzymes in post-mortem meat, and other - through microbial fermentation, chemical/enzymatic hydrolysis and with the help of proteolytic enzymes. The proteolytic enzymes can originate from animals, microorganisms or plants and what kind of proteolytic enzymes used will determine the types of bioactive peptides generated. The different substrates (blood, trimmings or bones) will also result in different bioactive peptides (Lafarga & Hayes 2014). 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 (Madhu et al. 2022). Another commonly used method to obtain bioactive peptides are microbial fermentation and *in vitro* digestion (Lemes et al. 2023). In this study, the focus will be placed on enzymatic hydrolysis with proteases. Bioactive peptides can also be generated through normal digestion of proteins in the gastrointestinal system of mammals (Adje et al. 2011).

5.1.1 Procedure description: Enzymatic hydrolysis with proteases

One major method to obtain bioactive peptides from waste and by-products from meat industry is to use proteolytic enzymes under controlled conditions such as pH and temperature. During this process, the proteins are hydrolysed and the bioactive compounds are released. Many proteolytic enzymes can be used, examples of commercial enzymes are pepsin, trypsin, chymotrypsin, corolase PP, papain, bromelain and pronase (Toldrá et al. 2021). These enzymes need different conditions to perform their action. A sequential hydrolysis is usually used, where multiple kinds of proteolytic enzymes are added at different times, often these enzymes have similar optimum conditions (Ulug et al. 2021).

Table 1. Some commercial enzymes (exo- and endo-peptidases) used for generation of bioactive peptides and their conditions needed for optimal functionality.

Enzyme name	EC number	Optimum temperature, °C	Optimum pH	Type, showing mechanism to cleave proteins	Origin
Papain	3.4.22.2	60-70	6-7	Cysteine protease	Papaya fruit
Bromelain	3.4.22.32	35-45	7	Sulfhydral protease	Pineapple fruit
Pronase	3.4.24.4	40-60	7.5	Non-specific protease	*Streptomyces griseus*

Papain has a temperature optimum of 60-70 °C and a pH optimum of 6-7 to effectively perform action and cleave proteins. Meanwhile, bromelain has a temperature optimum of 35-45 °C and a pH optimum of 7. Different enzymes needs different conditions to work and cleave proteins at different spots and this leads to generation of different bioactive peptides (Table 1) (Toldrá et al. 2021).

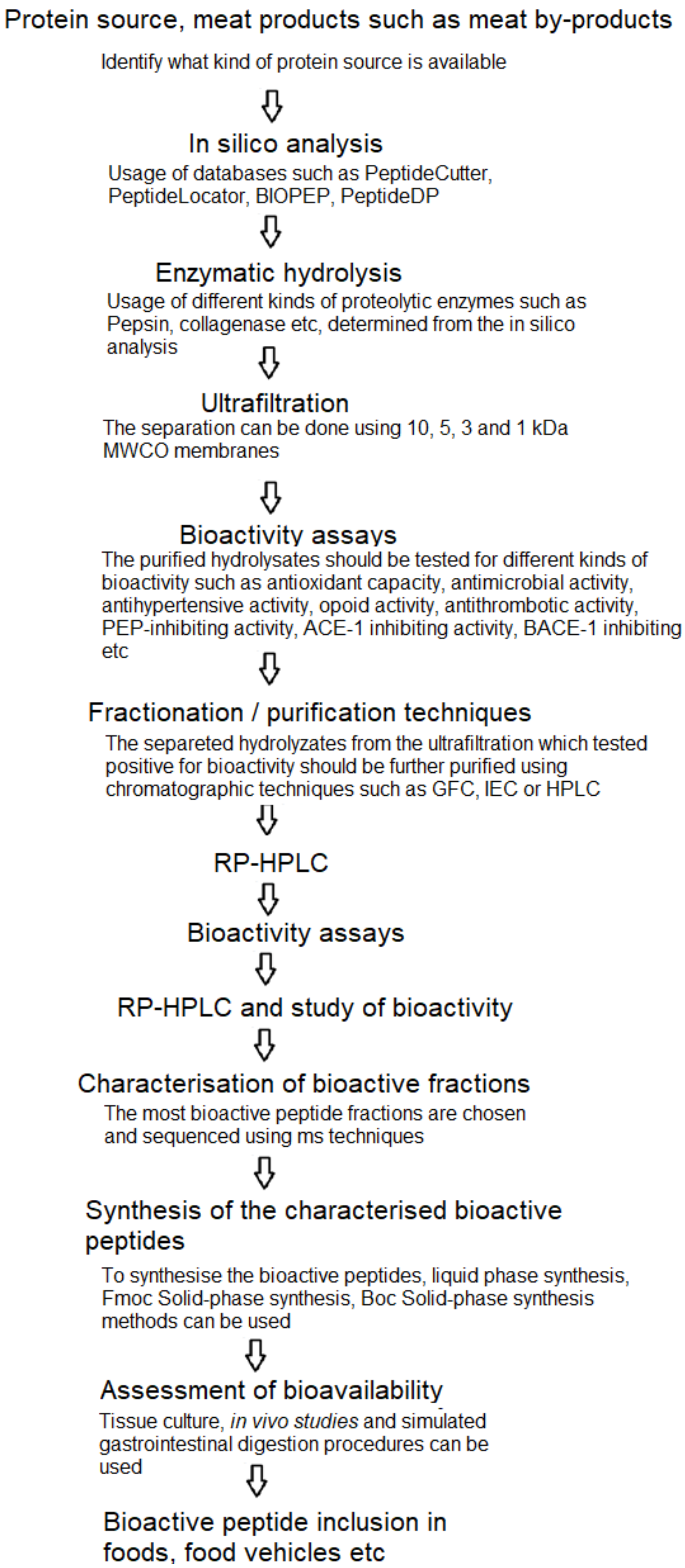


Figure 2. A flow diagram on generation of bioactive peptides from meat by-products.

Steps include generation, isolation, characterization and bioactivity assays. The first step in generation of bioactive peptides include a characterization of the meat products, what is the protein source. A in silico analysis should be carried out to characterize the protein source and the primary, secondary, tertiary and quaternary structure of the proteins. After the in silico analysis, the enzymatic digestion should take place, appropriate enzymes should be used for the specific protein source to generate the desired bioactive peptide (Minkiewicz et al. 2011). Many commercial proteolytic enzymes are available to use and combinations of those enzymes can be used, some of which are listed in table 1. The specific enzymes which should be used are determined by the in silico analysis to generate a desired hydrolysate (Cheung et al. 2009). The generated hydrolyzates are then purified by different kinds of separation techniques such as ultrafiltration. After the separation techniques the hydrolysates are tested for their bioactivities. The hydrolyzates that got a positive result from the bioactivity testing gets 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, these peptidic fractions are further tested for their bioactivity through bioactivity assays. The most bioactive peptide fractions are chosen and characterized using mass spectrometry techniques such as MALDI-TOF and ESI-MS (Lew 2004). The sequenced bioactive peptides are then synthesised for additional bioavailability studies. The synthesis of the bioactive peptides is usually done using liquid phase synthesis, Fmoc Solid-phase synthesis and Boc Solid-phase synthesis. Lastly dose response, safety and bioavailability studies in vivo should be done before inclusion of the bioactive peptide into a food product (Lafarga & Hayes 2014).

Low molecular extraction of the hydrolysates are necessary to be able to purify the bioactive peptides. The separation of peptides is an expensive and energy cost full procedure, therefore the use of electrodialysis with ultrafiltration membrane is commonly used in large scale production of bioactive peptides (Toldrá et al. 2021). The hydrolysate is a mixture of peptides and free amino acids, fractionation separetes the peptides in the hydrolysate into similar groups with respect to their mass, charge and hydrophobicity. Ultrafiltration is process applied in the beginning of fractionation to obtain peptides with a desired molecular weight from the hydrolysate. Chromatographic methods are also applied to purify certain peptides from the hydrolysate. The purification is based on the interaction between the mobile phase and the stationary phase. Size-exclusion chromatography, ion-exchange and reverse-phase high-performance liquid chromatography (RP-HPLC) can be used to fractionate different kinds of peptides from the hydrolysate. The chromatographic techniques are usually coupled with UV and fluorescence detectors. Mass spectrometry is commonly used to determine the peptide sequence (Ulug et al. 2021).

.

5.1.2 Advantages and disadvantages of enzymatic hydrolysis

At present, enzymatic hydrolysis is the most common method to generate bioactive peptides. The advantages of this method inlcudes its high specificity, mild conditions and absence of residual toxic compounds and organic solvents in the final product. The disadvantages of this method are the high cost of enzymes and the limited number of commercially available enzymes. Enzymatic hydrolysis is time-consuming and require a control over the temperature, pH, substrate and enzyme ratio which can be troublesome.

One of the reasons for the limited commercialization of peptide-based products is high cost of equipment. Moreover, this equipment cannot be used in industrial scale up. To solve these issues, tight collaboration between researchers and industrial partners are needed. The researchers can identify and characterize the bioactive peptides, whereas the industrial partners can apply this knowledge and own competences in production, product formulation, and consumer research (Kamioka et al. 2019).

6. Health effects of bioactive peptides

The activity of the bioactive peptide in the human body is determined by various aspects, including its amino acid sequence, weight, length, structure, hydrophobic/hydrophilic properties, and spatial structure of the peptide chain. Bioactive peptides may have many health effects. Examples include but not limited to antioxidant activity, anti-hypertensive activity, anti-inflammatory activity, antimicrobial activity, antitumor activity and opiod activity (Madhu et al. 2022).

6.1 Antihypertensive activity

High blood pressure (hypertension) is a risk factor for the development of cardiovascular diseases. Because of the increasing prevalence of such diseases, dietary approaches became an attractive option to prevent hypertension. Lowering of blood pressure is important in prevention of hypertension, which affects 20% of adults and can lead to stroke, myocardial infarction and heart failure. Renin-angiotensin system is responsible for controlling the blood pressure in humans. The system controls the blood pressure by converting angiotensin to angiotensin I, following converting of angiotensin I to angiotensin II by angiotensin I converting enzyme (ACE). The generation of angiotensin II causes vasoconstriction so by inhibiting the angiotensin I converting enzyme, the formation of angiotensin II is inhibited and thus no vasoconstriction occurs (Matsubara et al. 2006). In this regard, the study of anti-hypertensive ACE-inhibitory bioactive peptides are of great importance.

The GFPTTKTYFPHF and VVYPWT peptide sequences have been isolated with the help of proteomic techniques and showed 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 lowering the blood pressure.

Many of the anti-hypertensive bioactive peptides act as competitive inhibitors of the ACE enzyme. GFPTTKTYFPHF have an IC_{50} value of 4.9 μM and VVYPWT have an IC50 value of 6.0 μm from porcine blood. IC_{50} is value describing how much is needed to inhibit 50% of a given biological process, in this case the ACE enzyme.

6.2 Antitumoral activity

Bioactive peptides continue to attract attention in clinical tumor therapy. Bioactive peptides are characterised by high activity, low molecular weight, and easy trans-membrane absorption. Thus, these 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 acts through enhancing tumor cell apoptosis and inhibiting tumor angiogenesis (Xing et al. 2021).

Currently, several peptides with antitumoral activities were isolated from bovine meat (Jang et al. 2008), and many studies indicated the potency of meat waste and by-products as a source of antitumor peptides (Mora et al. 2014).

6.3 Antioxidant activity

Recently an increase in degenerative deceases was observed, likely due to an increased exposure to free radicals and reactive oxygen species. Bioactive peptides with antioxidant activity can inhibit the effect of free radicals and reactive oxygen species, as they can function as effective metal ion chelating agents and inhibit lipid peroxidation. Free radicals cause oxidative stress. Oxidative damage arises due to the fact that free radicals can chemically react with DNA, proteins and lipids. Oxidative stress can cause different types of cell damage and cell death. Antioxidants migh neutralize free radicals by donating electrons, which stabilizes the free radicals and makes them less reactive, thus inhibiting the free radical ability to react with other substances in the human body (López-García et al. 2022). Studies have shown that some antibiotics cause side effects such as oxidative damage. This has led to the search of new medicines or compounds with antioxidant properties, where bioactive peptides are an attractive option because they have no side effects and might be obtained from waste and by-products.

6.4 Opiod activity

Opiod activity is characterized by the interaction between opiod bioactive peptides and opiate receptors. Opiate receptors are transmembrane proteins receptors which are located in the brain. These receptors can act as both hormones and neuromodulators. Generally, opiod peptides can regulate many functions, including digestion, gastrointestinal motility, pancreatic secretion, stress and pain. Opiod peptides can be obtiained through the diet or can be synthezied by the body. Many opiod peptides were found in hemoglobin from bovine and from serum albumin from the blood. Cathepsin D is able to cleave proteins such as hemoglobin to hemorphin-4, -5 and -6. The hemorphins have opiod activity and can ease pain, inflammation, and can regulate blood pressure (Garreau et al. 1997). Studies have also shown that hemorphins can be promising pharmacological candidates to prevent neurodegenerative disease (Poljak et al. 2004). The hemorphin with the sequence of VVYPWTQ was able to inhibit the proliferation of tumor cells (Blishchenko et al. 2002).

6.5 Antimicrobial activity

Antimicrobial peptides can inhibit the growth of microorgansisms, such as gram-negative bacteria, gram-positive bacteria, viruses, fungi, and generally does not have any side effects. The side effects of many antibiotics have made antimicrobial peptides an attractive option for killing pathogens. They have a wide range of mechanisms to inhibit the growth of microorganisms. For example, the antimicrobial peptides might react with the membrane of the microorganism and create holes in it, and this destroys the microorganism. Jang et al (2008) identified four antimicrobial peptides with inhibitory activity against bacteria, *pseudomanas aeruginosa* from beef sarcoplasmic protein. The peptide GLSDGEQ showed inhibitory effects against gram-negative and gram-positive bacteria, *Salmonella typhimurium*, *Bacillus cereus*, *Eschericha coli* and *Listeria monocytogenes* (Madhu et al. 2022).

6.6 Immunomodulatory activity

Bioactive peptides with anti-inflammatory effects, have a great potential to act as anti-inflammatory agents and be used as therapeutic agents to treat inflammation disorders. Inflammation can be the first step to generate a sickness, such as skin disorder, cardiovascular syndrome or cancer (La Manna et al. 2018). A study was conducted where bioactive peptides from porcine muscle regulated the secretion of cytokines in a dose-related manner, and the cell viability increased when the peptide concentration increased (Xing et al. 2021). The increase in cytokines lead to a decrease in proinflammatory factors including IL-6 and TNF-α which in turn leads to a decrease in inflammation (Xing et al. 2021). A study conducted by Zhang et al (2010) showed that peptides from a chicken collagen hydrolysate reduced the secretion of interleukin-6, TNF-α and soluble intercellular adhersion molecule-1 by 43%, 24.1% and 17.9%. This reduced inflammation (Zhang et al. 2010).

7. Ongoing research in Sweden and Kazakstan on sustainable use of waste and by-products from meat industry

7.1 Sweden

Sweden is located in the north of Europe. Professor Anders Karlsson is a leading scientist in meat area, and responsible for a formas-financed project "Sensory sensation for both everyday and party – Use of side stream food resources to produce tasty, climate-smart and healthy meat products from nose to tail". He and Katarina, researcher at SLU shared their knowledge about currect situation with waste and by-products from meat industry and Sweden.

They explained, that approximately half of the by-products capable of becoming food from bovine and pig production, are not yet used. Under 2020, *1 100 00* tons of food waste was produced in Sweden, the households stood for the largest part (*Livsmedelsförluster vid slakt av grisar och nötkreatur* u.å.). The majority of the products are handled as waste and used for biogas production. Smaller portions are used as feed for companion animals, such as dogs and cats, and the rest is destroyed by incineration. The reason for the fact that almost half of the by-products are discarded, is the lack of profitable provision. An area of action can be to develop markets for these by-products, to develop export opportunities. Additionally, cost-effective ways to obtain the by-products, blood and guts should be developed. Blood is a by-product that is lost extensively. Cheek from cattle, and skull from pig is in great demand in the export market but not all slaughterhouses take advantage of that. Investments in product development, processing and marketing of products which originate from by-products will increase the proportion of by-products in Swedish markets and kitchens. This will help Sweden to come closer to many SDGs.

On a national level were 13 000 tonnes of by-products from cattle used for other purposes than food production. Blood stood for 5030 tonnes, liver - for 370 tonnes and cheek - for 260 tonnes.

Regarding pig production, 47% of total by-products used for other purposes than food production. In 2020, 227 000 tonnes of pig slaughter weight were produced.

The same year, 24 000 tonnes of pig by-products were used for other purposes than food production.

For large slaughterhouses, the majority of by-products that are not used for food production, are used for biogas production. Generally, larger slaughterhouses are using more by-products used for food purposes compared to middle large and small slaughterhouses. A reason for the low percent of by-products processed to foods by the middle large and small slaughterhouses are that they lack connections with trade partners. An option for them can be to sell the by-products to the larger slaughterhouses and let them to process them into food (*Livsmedelsförluster vid slakt av grisar och nötkreatur* u.å.).

The destruction of waste and by-products by incineration is cost-demaning. Therefore, minimizing the amount of waste and by-products has economical advantages.

7.2 Kazakstan

Republic of Kazakhstan is located in Central Asia, and traditions in Kazakhstan are very different compared to European countries. Nevertherless, issues with waste and by-products from meat industry is also important where. Government of Kazakhstan recently supported several research projects, which are aimed to turn waste and by-products to high-value materials. One of such projects is led by Assoc Professor Kadyrzhan Makangali from S.Seifullin Kazakh Agro Technical University. During the zoom meeting, Kadyrzhan informed about current situation in Kazakhstan and future plans. Bones and blood are not processed in Kazkahstan because of high costs and just discarded. However, several enterprises in Kazkahstan are already using by-products from meat industry to formulate animal feeds. Currently, Kadyrzhan is working on isolation of proteins from meat waste and by-products to use them in formulation of tasty and safe foods for elderly. "It is well known that elderly people might suffer from dysphagia (chewing and swallowing disorders), have a decline in their sensory perception and thus the nutritional intake may be insufficient. Our aim is to create a tasty, easy-to-eat and healthy product enriched with proteins, vitamins and minerals, which will help the older population to stay healthy. This will also help to achieve the SDGs in regard to end poverty, protect the planet, and ensure prosperity, and especially the SDG "good health and well-being" i.e., to ensure a healthy life and promote well-being for people of all ages", said Kadyrzhan.

8. Future perspectives and recommendations for further research

Bioactive peptides with health-promoting benefits derived from meat waste and by-products have a promising future in developing functional ingredients in foods and feeds. If they become commercialized, it will not only open up a new market with new products, but also greatly contribute to the achievement of SDGs. To transform waste and by-products from the meat industry (trimmings, skin, bones, fatty tissues, blood and internal organs) to high-value materials will contribute to global health of the consumers and provide economical benefits to producers. A range of new bioactive peptides with desirable bioactivities will open up broad opportunities in the food, feed and medicinal markets. Currently, research is mainly focused on peptide identification and characterisation as well as finding the optimal conditions for their isolation. To facilitate this process, novel sustainable technologies are needed. Such technologies should focus on minimal damage to the peptide structure, should not be time-consuming and have adequate costs (Ulug et al. 2021). One of the promising technique is microencapsulation of hydrolysates, which can be incorporated into foods, or can be sold as dietary supplements (Wang & Selomulya 2020; Sarabandi et al. 2020). Different methods to improve taste of certain peptides should also be developed and validated (Li-Chan 2015; Zhang et al. 2018). Moreover, future research should focus on the relationship between the chemical structure of bioactive peptide and its activity in the human body and potential interaction in different matrixes. Finally, more knowledge is needed on the health effects of bioactive peptides, including human and animals. Such knowledge is essential in the commercialization of the products, enriched with bioactive enzymes from waste and by-products from meat industry.

9. Concluding remarks

Meat consumption and production are actively debated nowadays. If meat production will continue to rise, more waste and by-products will be generated from the meat industry. Sustainable utilization of such products is a big step forward on the sustainability front. It helps fulfill many of the global development goals such as no poverty, zero hunger, climate action, decent work and economic growth, industry, innovation and infrastructure, responsible consumption and production, good health and well-being. This is especially important since the global malnutrition is on the rise. One of the promising methods that can utilize waste and by-products from the meat industry include isolation and further use of bioactive peptides through enzymatic hydrolysis. Bioactive peptides have a great potential as ingredients in functional food products to enhance their nutritional value. However, more research is needed to understand the health benefits of bioactive peptides, to optimize the methods of their isolation and incorporation of the food products, and to commertilise the products.

10. Acknowledgements

Special acknowledgements should be given to Anders Karlsson, Professor at the Department of Animal Environment and Health, SLU, and Kadyrzhan Makangali, Associate Professor at the S.Seifullin Kazakh Agro Technical University for their insightful guidance and tips. Also, to Galia Zamaratskaia, university lecturer and expert in structure and properties of food. I would also want to thank some of my colleagues for their help such as Nils Jönsson, Saga Preis and Sebastion Andersson Kurko.

References

Al-Gheethi, A., Ma, N.L., Rupani, P.F., Sultana, N., Yaakob, M. A., Mohamed, R. M.S. R. & Soon, C. F. (2021). Biowastes of slaughterhouses and wet markets: an overview of waste management for disease prevention. *Environmental Science and Pollution Research* https://doi.org/10.1007/s11356-021-16629-w

Adje, E.Y., Balti, R., kouach, M., Guillochon, D. & Nedjar-Arroume, N. (2011). α 67-106 of bovine hemoglobin: a new family of antimicrobial and angiotensin I-converting enzyme inhibitory peptides. *European Food Research and Technology*, 232 (4), 637–646. https://doi.org/10.1007/s00217-011-1430-z

Blishchenko, E., Sazonova, O., Surovoy, A., Khaidukov, S., Sheikine, Y., Sokolov, D., Freidlin, I., Philippova, M., Vass, A., Karelin, A. & Ivanov, V. (2002). Antiproliferative action of valorphin in cell cultures. *Journal of Peptide Science*, 8 (8), 438–452. https://doi.org/10.1002/psc.402

Cederberg, C. & Sonesson, U. (2011). *Global food losses and food waste: extent, causes and prevention; study conducted for the International Congress Save Food! at Interpack 2011, [16 - 17 May], Düsseldorf, Germany*. Gustavsson, J. (red.) (Gustavsson, J., red.). Rome: Food and Agriculture Organization of the United Nations.

Cheung, I.W.Y., Nakayama, S., Hsu, M.N.K., Samaranayaka, A.G.P. & Li-Chan, E.C.Y. (2009). Angiotensin-I Converting Enzyme Inhibitory Activity of Hydrolysates from Oat (Avena sativa) Proteins by In Silico and In Vitro Analyses. *Journal of Agricultural and Food Chemistry*, 57 (19), 9234–9242. https://doi.org/10.1021/jf9018245

Garreau, I., Fruitier, I., Sannier, F., Zhao, Q., Cucumel, K., Cupo, A. & Piot, J.-M. (1997). Identification of hemorphins in a cathepsin D bovine hemoglobin hydrolysate by radioimmunoassay and photodiode array detections. *Letters in Peptide Science*, 4 (4), 293–296. https://doi.org/10.1007/BF02442892

Kamioka, H., Tsutani, K., Origasa, H., Yoshizaki, T., Kitayuguchi, J., Shimada, M., Wada, Y. & Takano-Ohmuro, H. (2019). Quality of Systematic Reviews of the Foods with Function Claims in Japan: Comparative Before- and After-Evaluation of Verification Reports by the Consumer Affairs Agency. *Nutrients*, 11 (7), 1583. https://doi.org/10.3390/nu11071583

Karwowska, M., Łaba, S. & Szczepański, K. (2021). Food Loss and Waste in Meat Sector—Why the Consumption Stage Generates the Most Losses? *Sustainability*, 13 (11), 6227. https://doi.org/10.3390/su13116227

La Manna, S., Di Natale, C., Florio, D. & Marasco, D. (2018). Peptides as Therapeutic Agents for Inflammatory-Related Diseases. *International Journal of Molecular Sciences*, 19 (9), 2714. https://doi.org/10.3390/ijms19092714

Lafarga, T. & Hayes, M. (2014). Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. *Meat Science*, 98 (2), 227–239. https://doi.org/10.1016/j.meatsci.2014.05.036

Lew, R.A. (2004). HPLC in the analysis of peptide metabolism. *Methods in molecular biology (Clifton, N.J.)*, 251, 275–290

Li-Chan, E.C. (2015). Bioactive peptides and protein hydrolysates: research trends and challenges for application as nutraceuticals and functional food ingredients. *Current Opinion in Food Science*, 1, 28–37. https://doi.org/10.1016/j.cofs.2014.09.005

Livsmedelsförluster vid slakt av grisar och nötkreatur (u.å.). https://webbutiken.jordbruksverket.se/sv/artiklar/ra2218.html [2023-05-24]

López-García, G., Dublan-García, O., Arizmendi-Cotero, D. & Gómez Oliván, L.M. (2022). Antioxidant and Antimicrobial Peptides Derived from Food Proteins. *Molecules (Basel, Switzerland)*, 27 (4), 1343. https://doi.org/10.3390/molecules27041343

Madhu, M., Kumar, D., Sirohi, R., Tarafdar, A., Dhewa, T., Aluko, R.E., Badgujar, P.C. & Awasthi, M.K. (2022). Bioactive peptides from meat: Current status on production, biological activity, safety, and regulatory framework. *Chemosphere*, 307, 135650. https://doi.org/10.1016/j.chemosphere.2022.135650

Matsubara, K., Matsubara, Y. & Ito, M. (2006). Role of Renin-Angiotensin System in Vascular Endothelial Dysfunction of Pregnancy-Induced Hypertension. *Current Hypertension Reviews*, 2 (4), 311–316. https://doi.org/10.2174/157340206778742575

Minkiewicz, P., Dziuba, J. & Michalska, J. (2011). Bovine Meat Proteins as Potential Precursors of Biologically Active Peptides - a Computational Study based on the BIOPEP Database. *Food Science and Technology International*, 17 (1), 39–45. https://doi.org/10.1177/1082013210368461

Mora, L., Reig, M. & Toldrá, F. (2014). Bioactive peptides generated from meat industry by-products. *Food Research International*, 65, 344–349. https://doi.org/10.1016/j.foodres.2014.09.014

Novel technologies for the production of bioactive peptides | Elsevier Enhanced Reader (u.å.). https://doi.org/10.1016/j.tifs.2020.12.002

Patel, G. & Misra, A. (2011). 10 - Oral Delivery of Proteins and Peptides: Concepts and Applications. I: Misra, A. (red.) *Challenges in Delivery of Therapeutic Genomics and Proteomics*. London: Elsevier. 481–529. https://doi.org/10.1016/B978-0-12-384964-9.00010-4

Poljak, A., McLean, C.A., Sachdev, P., Brodaty, H. & Smythe, G.A. (2004). Quantification of Hemorphins in Alzheimer’s Disease Brains. *Journal of Neuroscience Research*, 75 (5), 704–714. https://doi.org/10.1002/jnr.20020

Sai, Y., Kajita, M., Tamai, I., Kamata, M., Wakama, J., Wakamiya, T. & Tsuji, A. (1998). Intestinal absorption of fluorescence-derivatized cationic peptide 001-C8-NBD via adsorptive-mediated transcytosis. *Bioorganic & Medicinal Chemistry*, 6 (6), 841–848. https://doi.org/10.1016/S0968-0896(98)00031-5

Sarabandi, K., Gharehbeglou, P. & Jafari, S.M. (2020). Spray-drying encapsulation of protein hydrolysates and bioactive peptides: Opportunities and challenges. *Drying Technology*, 38 (5–6), 577–595. https://doi.org/10.1080/07373937.2019.1689399

Toldrá, F., Reig, M. & Mora, L. (2021). Management of meat by- and co-products for an improved meat processing sustainability. *Meat Science*, 181, 108608. https://doi.org/10.1016/j.meatsci.2021.108608

Ulug, S.K., Jahandideh, F. & Wu, J. (2021). Novel technologies for the production of bioactive peptides. *Trends in Food Science & Technology*, 108, 27–39. https://doi.org/10.1016/j.tifs.2020.12.002

Wang, Y. & Selomulya, C. (2020). Spray drying strategy for encapsulation of bioactive peptide powders for food applications. *Advanced Powder Technology*, 31 (1), 409–415. https://doi.org/10.1016/j.apt.2019.10.034

Xing, L., Li, G., Toldrá, F. & Zhang, W. (2021). Chapter Four - The physiological activity of bioactive peptides obtained from meat and meat by-products. I: Toldrá, F. (red.) *Advances in Food and Nutrition Research*. Academic Press. 147–185. https://doi.org/10.1016/bs.afnr.2021.02.016

Zhang, C., Alashi, A.M., Singh, N., Liu, K., Chelikani, P. & Aluko, R.E. (2018). Beef Protein-Derived Peptides as Bitter Taste Receptor T2R4 Blockers. *Journal of Agricultural and Food Chemistry*, 66 (19), 4902–4912. https://doi.org/10.1021/acs.jafc.8b00830

y.com/browse/waste [2023-04-20]

Meyer, C., Thiel, S., Ullrich, U., & Stolle, A. (2010). Salmonella in raw meat and by-products from pork and beef. Journal of food protection, 73(10), 1780–1784. https://doi.org/10.4315/0362-028x-73.10.1780

Ryder, K., Bekhit, A.el-D., McConnell, M., & Carne, A. (2016). Towards generation of bioactive peptides from meat industry waste proteins: Generation of peptides using commercial microbial proteases. Food chemistry, 208, 42–50. https://doi.org/10.1016/j.foodchem.2016.03.121

Ulug, S.K., Jahandideh, F., & Wu, J. (2021). Novel technologies for the production of bioactive peptides. Trends in Food Science and Technology, 108, 27-39.

Wikipedia (2022). File:Sustainable Development Goals.jpg. https://commons.wikimedia.org/wiki/File:Sustainable_Development_Goals.jpg

Xing, L., Li, G., Toldrá, F. & Zhang, W. (2021). Chapter Four - The physiological activity of bioactive peptides obtained from meat and meat by-products. I: Toldrá, F. (red.) *Advances in Food and Nutrition Research*. Academic Press. 147–185. https://doi.org/10.1016/bs.afnr.2021.02.016

Zhang, Y ., Kouguchi, T ., Shimuzu, M., Ohmori, T ., Takahata, Y., & Morimatsu, F. (2010). Chicken collagen hydrolysate reduces proinflammatory cytokine production in C57BL/6.KOR-ApoEshl mice. Journal of Nutritional Science and Vitaminology, 56(3), 208-213.

Zhao, J., Zhang, X., Liu, H., Brown, M.A. & Qiao, S. (u.å.). Dietary Protein and Gut Microbiota Composition and Function. *Current Protein & Peptide Science*, 20 (2), 145–154

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. You will find a link to SLU's publishing agreement here:

- https://libanswers.slu.se/en/faq/228318.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.