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SAFETY ASSESSMENT OF ANIMAL OFFAL AND ITS PROCESSING INTO PROTEIN HYDROLYSATES FOR THE DEVELOPMENT OF FUNCTIONAL FOOD PRODUCTS

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CONTENTS

ABBREVIATIONS

AA	–	amino acid
$ABTS^{\bullet+}$	–	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity
Ag	–	agar
Ala	–	alanine
Arg	–	arginine
Asp	–	aspartic acid
B. cereus	–	latin *Bacillus cereus* ATCC 11778 – Gram-positive, rod-shaped bacteria, facultative anaerobes
C3	–	Category 3 animal by-products
$DPPH^{\bullet}$	–	2,2-diphenyl-1-picrylhydrazyl radical scavenging activity
E	–	edible offal
EFSA	–	European Food Safety Authority
E. coli	–	latin *Escherichia coli* ATCC 25922 – Gram-negative, rod-shaped bacteria, facultative anaerobes
EU	–	European Union
FA	–	fatty acid
GC	–	gummy candy
GI	–	gelatine
Glu	–	glutamic acid
Gly	–	glycine
h/H	–	hypocholesterolaemic/hypercholesterolaemic ratio
His	–	histidine
Ile	–	isoleucine
L*, a*, b*	–	color metrics, used to describe and quantify the color (lightness (L*), redness (a*), and yellowness (b*))
LC–MS/MS	–	liquid chromatography–tandem mass spectrometry
L. monocytogenes	–	latin *Listeria monocytogenes* ATCC 13932 – Gram-positive, rod-shaped bacteria, facultative anaerobes
LST	–	Lithuanian standard
MCFA	–	medium-chain fatty acids
Met	–	methionine
min	–	minute
MUFA	–	monounsaturated fatty acids
n.s.	–	not significant
OA	–	overall acceptability
Pa	–	papain
Pb	–	lead
Pe	–	pepsin
Phe	–	phenylalanine
Pro	–	proline
PUFA	–	polyunsaturated fatty acids
RP	–	reducing power

S. Typhimurium	–	latin *Salmonella enterica* serovar Typhimurium ATCC 14028 – Gram-negative, rod-shaped bacteria, facultative anaerobes
S. aureus	–	latin *Staphylococcus aureus* ATCC 25923 – Gram-positive, spherical (cocci-shaped) bacteria, facultative anaerobes
SCFA	–	short-chain fatty acids
Ser	–	serine
SFA	–	saturated fatty acids
SWATH-MS	–	sequential window acquisition of all theoretical mass spectra
TAMC	–	total aerobic microbial count
TFA	–	total fatty acid
Thr	–	threonine
TI	–	index of thrombogenicity
Tyr	–	tyrosine
UFA	–	unsaturated fatty acids
VA	–	Veterinary Academy
Val	–	valine

INTRODUCTION

As the world's population continues to grow – estimated at 8.2 billion in 2024 and projected to reach 9.7 billion by 2050 – there is increasing focus on sustainable and nutritious sources of protein and the efficient use of existing biological resources [1]. Globally, food loss and waste represent one of the major inefficiencies in modern food systems, leading to significant environmental, economic, and nutritional impacts [2]. The Food and Agriculture Organization estimates that approximately 1.3 billion tons of food are lost or wasted worldwide annually [3]. In the meat processing industry, numerous by-products such as offal, bones, and blood are often underutilized or discarded [4]. This underutilization is partly explained by the fact that the live weight of animals is divided into edible tissues and inedible parts, of which by-products account for about 66% of the slaughter weight of bovine, 52% of ovine, and up to 80% of porcine [5]. The liver is one of the most commonly rejected organs after post-mortem analysis, and its pathologies account for the largest economic loss (58.64%) [6]. This not only results in the loss of valuable nutrients but also contributes to environmental challenges, including increased greenhouse gas emissions, higher water and land use, and pollution associated with improper waste management [7]. Animal by-products, especially liver and heart, are rich in nutrient-dense and bioactive compounds, yet they remain underutilized despite their high nutritional value, making them a promising raw material for the production of functional ingredients [8]. Consequently, the valorisation of animal-derived biomass has become a key priority, as the recovery of valuable compounds from meat by-products is increasingly recognized as an effective strategy to reduce waste and improve nutrient utilization [9, 10]. However, despite their biochemical richness, the valorisation potential of these organs remains insufficiently explored.

According to the European Fat Processors and Renderers Association (EFPRA), meat processing plants generate approximately 5 million tonnes of Category 1 and 2 animal by-products and about 12 million tonnes of Category 3 by-products annually [11]. However, a significant share of Category 3 slaughterhouse by-products is classified and exported as Category 1 material, thereby reducing their economic and nutritional potential [12]. As a result, these raw materials are often diverted to non-food uses, despite being rich in high-biological-value proteins with considerable nutritional and nutraceutical potential for human diets. It is estimated that using even half of these resources could yield an additional 200 million kilograms of protein per year [13]. The use of Category 3 slaughterhouse by-products is limited due to strict regulatory requirements and safety restrictions, which limit their application

[12, 14]. Despite their significant nutritional and functional potential, Category 3 slaughterhouse by-products remain understudied and underutilized, as current methods for processing and valorization of these secondary meat raw materials are not sufficiently developed and widely applied in practice [4].

These combined environmental and public health concerns highlight the need to re-evaluate the use of meat by-products, considering them not as waste but as valuable raw materials. According to the European Fat Processors and Renderers, enzymatic protein digestion, a fraction of meat by-products, allows for the extraction of bioactive molecules and creating high-value-added ingredients [15, 16]. This improves their bioavailability and functional properties, such as solubility, viscosity, emulsification and gel formation, thus enabling the extraction of bioactive compounds and the development of high-value-added ingredients [17, 18]. Animal-derived by-products are recognised as promising raw materials for producing bioactive hydrolysates, as the compounds they contain exhibit significant physiological properties and perform important biological functions in the human body [17]. The biochemical composition of organ-derived by-products differs between species: porcine liver and heart are rich in metabolic enzymes, heme-containing proteins and bioactive compounds, whereas bovine organs contain higher levels of structural proteins and connective-tissue components, while ovine organs exhibit a distinctive proteomic and amino acid profile [19, 20]. These interspecies differences influence substrate availability for enzymatic cleavage and consequently shape the peptide profiles generated during hydrolysis [21].

Due to their high protein content, meat by-products represent an excellent substrate for generating peptides with diverse biological activities [22, 23]. Bioactive peptides formed during enzymatic hydrolysis of larger protein molecules may display antioxidant, antimicrobial, antihypertensive, anti-inflammatory, and immunomodulatory properties [24, 25]. Due to these properties, peptides have received increasing attention in recent years as physiologically active molecules with therapeutic value. Their high specificity contributes to safety, better tolerability and efficacy, and therefore they are considered promising candidates in modern drug development practice [22]. The conversion of meat by-products into bioactive peptides can significantly reduce waste streams, thereby lowering environmental impacts and improving the sustainability of meat production systems [26]. The growing burden of metabolic diseases and gut-related health disorders further emphasizes the need for new nutritional and dietary interventions capable of modulating the gut microbiota and enhancing public health [13, 18]. Bioactive peptides derived from meat by-products influence the balance of the intestinal microbiota, strengthen intestinal barrier integrity, and positively modulate immune

function, making them suitable as functional additives in therapeutic diets [27]. Moreover, integrating bioactive peptides derived from meat by-products into functional foods or dietary supplements could unlock significant yet largely unrealised economic opportunities, particularly in regions where the meat industry is substantial but waste management systems remain underdeveloped [15]. However, despite increasing scientific interest, current knowledge on the efficiency of peptide extraction, the optimisation of enzymatic hydrolysis conditions, and the functional integration of resulting hydrolysates into food systems remains fragmented and insufficiently developed [16].

The quality, safety, and functionality of offal and Category 3 animal by-products remain essential factors for their effective utilization in food systems. The lack of standardized methods for assessing Category 3 post-mortem lesions may limit the accurate classification of these raw materials and complicate their efficient selection for further use and processing. Therefore, to ensure their safe and effective utilization, a comprehensive evaluation of pathological changes, biological, chemical, and microbiological safety, as well as nutritional value, is necessary.

The increasing focus on sustainable resource utilization and waste reduction highlights the potential of offal and Category 3 animal by-products for the extraction of bioactive compounds and the development of higher value-added products. Through enzymatic hydrolysis, sufficiently stable peptides with potential antioxidant and antimicrobial activity can be obtained. The application of these peptide hydrolysates may be promising for the development of functional ingredients and innovative food products, while ensuring more sustainable processing of animal-derived raw materials in the meat industry.

The aim of the study

To assess the safety of animal offal and its suitability for processing into protein hydrolysates and the development of functional food products.

Objectives of the study

1. To evaluate pathological lesions in offal (porcine, bovine, and ovine) identified during veterinary post-mortem inspection and assess their impact on biological and chemical safety, nutritional composition, and the suitability of the raw materials for further processing.
2. To apply an optimal enzymatic hydrolysis model to selected offal to obtain protein hydrolysates with functional properties.

3. To evaluate the antioxidant and antibacterial properties and peptide profiles of offal hydrolysates, and to assess their potential application in the development of functional food products.
4. To develop a prototype of a novel product, evaluate its safety, nutritional composition, and functional properties, and make evidence-based decisions regarding the use of offal in the development of functional food products.

Scientific novelty

This study investigates underutilized raw materials – Category 3 animal by-products and edible offal – characterized by high biological value as sources of high-quality proteins and bioactive compounds. For the first time, a comprehensive comparative assessment of these raw materials was conducted, emphasizing their safety, nutritional value, and technological applicability in food systems.

The selected raw materials were analyzed according to the most important biological, chemical, microbiological safety, and pathological lesions aspects, thereby determining their suitability for further processing. Bioactive peptide hydrolysates with antioxidant, antimicrobial, and functional properties were obtained through enzymatic hydrolysis, with the influence of enzyme type and degree of hydrolysis on the biological activity of the hydrolysates evaluated in detail. Peptide sequence analysis enabled the identification of peptides with potential biological activity and revealed their applicability in the development of functional food products.

The peptide hydrolysates derived from livestock by-products were incorporated for the first time as functional additives into gummy matrices, resulting in the development of innovative food product prototypes.

The results of this study provide new scientific knowledge regarding the application potential of Category 3 animal by-products and edible offal, highlighting their importance as sustainable and economically valuable raw materials for the development of functional food ingredients and products.

1. LITERATURE REVIEW

1.1. Animal by-products, possibilities of their processing

The slaughter of animals for food generates substantial amounts of non-meat biomass, which often has limited commercial value and, if not properly managed, can result in high disposal costs [28]. In the European Union (EU) [29], offal is defined as fresh meat separated from the carcass and consisting of internal organs and blood, while excluding skeletal muscle tissue.

This category includes smooth muscle tissue and viscera – liver, kidney, heart, tongue, pancreas, spleen, brain, intestines and lungs – as well as blood; all of these parts can be used as food ingredients [30–34]. Offal is also classified according to its characteristic colour. Red offal includes liver, heart, kidney, tongue, spleen and testicles [35]. White offal, which usually requires extensive heat treatment, in contrast, includes lungs, spleen, thymus, intestines, brain and tail [36]. There is also a group of black offal, which includes the head, tibia, neck and hooves [34, 37]. Meat by-products can constitute up to 40proc. of the total carcass weight and are characterised by high nutritional value, being rich in high-quality proteins, vitamins, macro- and microelements, fats, and bioactive peptides, whose amounts are often comparable to or even higher than those found in muscle tissue [5, 38–41]. The slaughtering of livestock in abattoirs generates a significant amount of by-products that can be used for human consumption or processed into secondary products for agricultural and industrial applications [42]. In developed countries, these by-products are estimated to represent about 10–15% of the live animal's total value. However, they account for approximately two-thirds of the animal's total mass after slaughter [5].

The meat sector has limited options for utilizing waste materials due to the stringent criteria outlined in Regulations (EC) No. 999/2001 and No. 853/2004. Based on their degree of danger, animal by-products are divided into three categories (1, 2, and 3) by Council Directive 75/442/EEC. The only materials deemed safe for use in the manufacture of food and feed are those classified as Category 3. Category 3 animal by-products are not intended for human consumption and generally do not enter the food chain due to commercial factors such as limited demand and low economic value [43]. Current legislation allows the use of these materials in animal feed or the export of organs to countries where they are traditionally consumed [44]. Therefore, under current legal regulations, the use of Category 3 animal by-products is mainly limited to their use in animal feed and export to markets where their consumption is culturally acceptable.

The global utilisation of animal by-products is expected to continue to increase, as these materials possess high nutritional value and can help address the growing challenge of resource depletion [45, 46]. Meat by-products have often been neglected in dietary guidelines and recommendations, even though they generally provide nutrients with higher bioavailability [45–47]. As a result, consumers today know little about its nutritional value. The quality of edible offal is determined by numerous factors, including species, age, sex, live weight, fat content, and housing conditions, as well as genetic and environmental factors, such as the animal's diet and housing system [47–50]. Their chemical composition is similar to that of meat, being rich in proteins, amino acids, and fatty acids [34, 51]. Despite the high nutritional value of offal, its utilization is still low [52, 53]. Although these products are widely used in various dishes and food products worldwide [6, 14–16]. Their consumption is limited by cultural traditions, social habits, and consumer attitudes [30, 54, 55]. In developed countries, by-products are consumed very rarely, which is associated with a wide range of other food options and changing taste and consumption habits [56].

Across animal species, metabolically active organs such as the liver and heart have the highest protein concentrations [57]. In bovine offal, liver tissue typically contains about 18–20% protein, porcine liver about 17–19%, and ovine liver 18–20% [58–60] . In general, bovine offal tends to have a slightly higher and more stable protein concentration than porcine and ovine offal, whereas porcine offal is more variable in lipid content, which can reduce the relative protein content [61]. Ovine offal has a similar nutritional value to bovine offal, although it is used less in the food industry, mainly due to regional consumption patterns and lower commercial demand [62].

Meat by-products are rich in protein, ranging from about 7% in intestines to about 30% in the liver of animals [57]. Differences in the amount and type of amino acids arise from variations in the protein composition of by-products, such as their collagen content [10]. Recently, the meat industry has increasingly focused on liver, mainly due to its high protein concentration (18.54%), low fat content (3.38%), and rich phospholipid content, which is beneficial to human health [63]. Particularly appreciated is the liver's amino acid profile, which is rich in essential (tyrosine and phenylalanine) and hydrophobic (leucine, valine and isoleucine) amino acids with strong antioxidant properties [57]. The highest levels of essential amino acids – including threonine, valine, isoleucine, leucine, phenylalanine, lysine, and histidine – have been found in porcine and ovine livers, where they account for nearly 50% of the total amino acid content [45, 56].

Meat by-products, liver, heart, kidneys, and lungs – exhibit minimal fat content [35, 46]. Meat by-products are a valuable source of various fatty

acids. It contains both saturated (SFA) and unsaturated fatty acids; however, monounsaturated fatty acids (MUFA) generally predominate, while the proportion of polyunsaturated fatty acids (PUFA) is lower [46]. Meat by-products serve as a source of essential fatty acids such as linoleic acid (C18:2n-6) and alpha-linolenic acid (C18:3n-3), as well as polyunsaturated fatty acids like arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-3), which play a crucial role in preventing chronic diseases, including ischemic heart disease and certain types of cancer [57].

Meat by-products are especially rich in folic acid, choline, and vitamin B_{12}, offering a level of bioavailability rarely found in other foods [33]. However, these products are highly perishable, and their effective use requires the application of efficient moisture-reduction and preservation techniques [64].

1.2. Post-mortem analysis of animal organs and the prevalence of their lesions

Post-mortem inspection of organs is usually carried out using traditional methods such as gross visual inspection, palpation, sectioning and olfactory analysis, using the senses of sight, touch and smell [6]. The procedures are regulated by European Union legislation – Official Controls Regulation (EU) No 2017/625 and Commission Implementing Regulation (EU) No 2019/627 [65, 66]. In each case where a violation is detected during post-mortem analysis, appropriate rejection criteria are applied to ensure that meat and meat by-products unfit for human consumption do not enter further processing [6] .

Post-mortem examinations conducted in Lithuania showed that pathological changes were detected in 82.24% of porcine and 64.49% of bovine. Liver changes were most frequently detected in both species: in porcine, they accounted for 31.79%, and in bovine, 25.09%. Kidney and heart lesions were detected less frequently: in porcine, 8.87% and 6.23%, respectively, in bovine – 9.60% and 8.41% [67].

The most common pathology in porcine was pleurisy (10.2%), followed by pneumonia (8.5%), lesions (5.0%), milky turgor (3.8%), and pericarditis (3.3%) [34]. Post-mortem examination data from bovines show a similar pattern of pathological findings. Lesions were associated with lung disease, cardiac (pericarditis), steatosis, and renal pathologies, reflecting the most common respiratory, circulatory, and metabolic disorders [68]. Previous studies indicate that the most common pathological lesions in ovine are found in the liver (51.0%) and lungs (48.7%), while their prevalence in the heart

(3.9%) and kidneys (0.65%) is considerably lower. The most frequent causes of organ condemnation were associated with pneumonia and fasciolosis [69].

Respiratory tract diseases are among the most common and complex pathologies diagnosed in animals [70]. Pleurisy and pneumonia are the most common pathological lesions detected during post-mortem inspection [71]. Pneumonia is an inflammation of the lungs caused by viruses and bacteria, especially *Mycoplasma hyopneumoniae* and *Pasteurella multocida* [72]. Pleurisy is an inflammation of the pleura that can occur in acute or chronic form. Chronic pleurisy is usually characterised by abscess formation, fibrosis, and adhesion of the lung to the chest wall [73]. Most often, this disease develops as a complication of inflammation of other thoracic organs, especially the lungs, although in some cases the pericardium is also affected [74] .

Post-mortem liver lesions are quite common and lead to complete organ rejection. The frequency of liver condemnation due to pathological lesions varies significantly between different species of fattening animals. According to the frequency, they are arranged in the following order: heifers (14.17%), fattening bulls (7.97%), fattening porcine (11.26%), and ovine (4.73%) [75]. In previous studies, it was found that the most common liver lesion in cattle was steatosis, detected in 48.5% of cases, followed by abscesses (23.2%) [68]. Both pathologies are typical for high-yielding cows, as hepatic steatosis is associated with a metabolic imbalance that usually develops in early lactation, when energy demand exceeds energy intake with feed, and fat oxidation in the liver is insufficient for proper lipid metabolism. As a result, fats accumulate in hepatocytes, causing fatty degeneration of the liver and impaired liver function, ultimately compromising the metabolic homeostasis and productivity of the animal [76]. The most common liver lesions observed in porcines were the so-called “milk spots”, often associated with ascariasis, but they can also be a consequence of changes caused by hepatitis, perihepatitis, necrotic and cirrhotic processes [77–79].

Pericarditis is the main cause of heart rejection [79]. It is a non-specific pathological condition that most commonly results from infectious processes, which may be of viral or fungal origin but are predominantly bacterial. In porcine, pericarditis is most frequently associated with infections caused by *Haemophilus parasuis*, *Pasteurella* spp., *Mycoplasma* spp., and *Streptococcus* spp. [79, 80].

Kidney lesions were the least frequently detected during post-mortem examinations in Lithuanian slaughterhouses, and the differences in their prevalence between different animal species were minimal – they amounted to 8.9% in porcine and 9.6% in bovine [67]. Previous studies have noted that the prevalence of kidney damage is highest in bovine (40.46%), followed by

porcine (28.45%), and lowest in goats and ovine (2.68% and 3.01%, respectively). The low prevalence of kidney lesions in ovine and goats is thought to be associated with their feeding [75, 81]. These species mainly graze on natural pastures and are characterized by selective feed selection, which allows for more effective regulation of metabolic load and thus reduces the risk of kidney damage [81]. The most commonly identified pathologies in the kidneys are renal cysts, hydronephrosis, pyelonephritis, the so-called "white-spotted kidneys", and urolithiasis [82].

1.3. Safety of animal by-products

1.3.1. Biological safety

The main reasons for the disposal of animal organs in slaughterhouses are related to parasitic and inflammatory diseases. The frequency of parasitic lesions depends on the species' susceptibility to various parasites and the animals' age. A comparison between adult and fattening animals in the Czech Republic showed a significantly higher incidence of parasitic damage in heifers (1.31%) compared to cows (0.69%) and fattening bulls (0.51%), as well as in finishing porcine (3.68%) relative to sows (0.58%). Conversely, a significantly higher number of lesions was observed in ewes (7.51%) compared to lambs (3.51%), and in goats (0.74%) compared to kids (0.18%) [75]. Animal organs are most commonly affected by endoparasites, mainly roundworms (nematodes), which usually parasitize the digestive tract (including the lungs), and flatworms (trematodes), such as liver flukes and tapeworms. Studies have shown that these parasites can cause various pathological changes, including fasciosis, peritonitis, hydatidosis and emphysema [75, 83].

Liver damage in animals is often associated with parasitosis, where parasites directly damage the liver, acting as a target organ, or indirectly, when the liver becomes a migration route for parasites, both during normal and accidental migration within the body [84]. Parasitic liver lesions are most commonly detected in grazing animals with a higher likelihood of parasitic invasion – ovine (7.51%), lambs (3.51%), and heifers (1.31%) – as well as in animals with limited antiparasitic protection due to meat safety requirements regarding antiparasitic residues, such as finishing porcines (3.68%). In comparison, they are rarely observed in rabbits and poultry (< 1%) [75]. The most common lesion of the porcine liver described in previous studies is the so-called "milk spot liver" [85] . This term is used to describe white spots of fibrosis in the liver parenchyma, which are formed due to an inflammatory reaction caused by the migration of *Ascaris suum* larvae [86]. These lesions

are typically asymptomatic and are only detected during post-mortem veterinary inspection in slaughterhouses, where the presence of milk spots is considered the main reason for liver condemnation by official veterinarians [78, 79]. During post-mortem examination, parasitic diseases such as parasitosis caused by *Echinococcus granulosus*, *Fasciola* spp. And *Dicrocoelium dendriticum* were most commonly detected in the liver of ruminants [87]. Cystic echinococcosis caused by *Echinococcus* spp. Is characterized by fluid-filled cysts in which numerous secondary (daughter) vesicles form [88]. These cysts develop in the internal organs of infected intermediate hosts, including domestic animals such as ovine, bovine, and porcine [88, 89]. Although the liver and lungs are most commonly affected, less frequently, foci of disease are found in the spleen, heart, kidneys, or brain [88, 90].

Like echinococcosis, fasciolosis is a significant parasitic disease affecting the liver and bile ducts of livestock. Fascioliasis is a parasitic liver disease caused by the trematode *Fasciola hepatica*, which involves the liver and bile ducts of bovine [91, 92]. This parasitosis is one of the most common post-mortem pathologies in the liver of bovine and ovine, characterized by characteristic macroscopic changes such as parenchymal fibrosis, thickening of the bile duct walls, migration marks on the liver surface, and accumulations of dark brown pigments (hematin) resulting from the metabolism of the parasites [69, 93–95].

Heat treatment is considered one of the most effective methods for controlling parasites in meat. However, its effectiveness depends on the specific parasite species, its developmental stage, and the temperature and duration of exposure [96]. Heat treatment of meat to 60–75 °C or freezing to −21 °C are sufficiently effective methods that can kill most parasites in animal products [97].

Contamination of viscera with microorganisms can occur during slaughter, especially during evisceration, when poor hygiene conditions promote microbial spread and significantly reduce shelf life. One of the main causes of contamination is contact between red and white viscera [98]. Most frequently, pathogenic germs contaminate carcasses during the slaughtering process, such as when the carcass is skinned, eviscerated, or chilled [99]. Previous studies have shown that visceral organs (kidneys, liver, and heart) have lower APC levels than other organs. Given the extent of processing, one would expect these organs to have similar bacterial counts to meat. The heart, kidney, and liver are generally considered nearly sterile organs at the time of dissection. Still, contamination can occur during the processing process when these organs are cross-contaminated with other products or during packaging stages [100]. Vanderlinde et al. [43] showed that the microbiological quality of meat by-products is influenced by the type of offal and the species of

animal. It was found that the total aerobic bacterial count (APC) and the prevalence of *Escherichia coli* were higher in non-organic offal, such as tongue or tendon, compared to meat [43].

Among the pathogens that can be introduced through cross-contamination, the most commonly identified pathogens are: *Salmonella* spp., *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* [101]. When analyzing microbial contamination, it was observed that the prevalence of *E. coli* O157 and *S. aureus* bacteria in the liver was higher when the workload of workers was more intense; therefore, strict application of hygiene norms and ensuring food safety standards in slaughterhouses are essential factors to guarantee the safety of meat by-products [102]. Due to concerns about contamination of bovine carcasses and by-products with pathogens such as *Salmonella* spp. And pathogenic *E. coli*, it is recommended to avoid palpation and incision during post-mortem inspection to reduce the risk of spreading these significant biological hazards, as referred to in Regulation (EU) 2019/627 [66].

1.3.2. Chemical safety

Pesticides are among the main sources of environmental contamination [103]. Over a thousand of these chemicals are classified as organochlorines, organophosphates, carbamates, and synthetic pyrethroids [104]. These compounds build up in the tissues of animals and plants after entering the food chain through soil, water, air, and sediments. At higher trophic levels of the food chain, their concentrations gradually rise [105–108]. Previous studies have shown that organochlorine pesticides (OCPs) are most commonly found in animal fat, especially in bovine and ovine. Although pesticide and PCB concentrations in fat are generally higher than in meat and offal due to their lipophilicity, most values did not exceed the permissible limits set by the European Union [104]. Most pesticides have the same maximum residue limits (MRLs) for different raw materials. For example, the European Union (EU) has set an MRL of 1.0 mg/kg for DDT (sum of all isomers and metabolites) in animal muscle, fat, liver, kidney, and other edible products. Furthermore, previous studies have shown that pesticide transfer coefficients may vary between different animal species, such as bovine, ovine, porcine, poultry, etc., due to differences in liver and kidney fat content [109]. For example, overfed geese have been shown to accumulate more liver fat than bovine or ovine, and therefore, their bioaccumulation and transfer coefficients of lipophilic pesticides may be significantly higher [110–113].

While intricate physiological processes influence their distribution within the animal tissues, the bioaccumulation of pesticides in animal-derived

products is dependent on tissue composition, specifically lipid and water content, with higher concentrations usually found in fatty tissues and the liver [114, 115]. Numerous severe illnesses, including cancer, neurological conditions, respiratory conditions, diabetes, and endocrine system disturbances resulting in reproductive and developmental disorders, are linked to long-term exposure to pesticides [116]. Regulatory authorities worldwide establish maximum residue limits (MRLs) for pesticides in animal products and their byproducts. The European Union (EU) consistently regulates pesticide residues, not only in meat and milk, but also in by-products such as liver, kidney, fat, and other tissues, from food production or processing [117].

According to scientific research, the distribution of pesticide residues in bovine tissues is varied, with the liver usually exhibiting the greatest quantities, followed by the tongue, muscle, and kidney tissues [118]. Since the liver is the main organ responsible for the metabolism and accumulation of pesticides, increased levels of pesticides in the liver may be related to its detoxification function [119, 120]. The liver, as the body's main filtering and detoxifying organ, tends to accumulate pollutants, especially polychlorinated biphenyls (PCBs) [121].

The presence of heavy metals in by-products is one of the primary issues with their condemnation. Due to their chemical stability and lack of biodegradability, these elements can build up in biological systems and the environment over time [122]. They interfere with regular cellular processes by attaching to structural proteins, enzymes, and nucleic acids. Because of their lengthy biological half-life and capacity to build up in both human and animal cells, they can have long-term harmful consequences. Heavy metals can enter animals through several routes, including inhalation of polluted air and consumption of contaminated water or plants [123]. Industrial activities, such as coal burning and metal smelting in grazing areas, can also contribute to animal health problems and product contamination [124].

Metals such as Cd, Cr, Pb, As, and Hg are harmful even in trace concentrations. They alter human metabolic systems, offer major health concerns, and can result in cancer, birth deformities, central nervous system damage, and neurological problems [125]. The levels of heavy metals in meat by-products are variable, including time of year, location, and body organ tested [126]. Heavy metals are neither degraded nor destroyed by the body, so they tend to accumulate in biological tissues, particularly in the muscle, kidney, and liver. However, these tissues also participate in detoxification processes and are therefore considered primary targets for heavy metal analysis [127]. Livestock, including bovines, are frequently employed as bioindicators of heavy metal pollution in the environment, offering information on how these metals build up in ecosystems [128]. Cadmium levels

in the soil and feed, as well as the age of the animals, have an impact on high cadmium concentrations in the kidneys of cattle. While cadmium concentrations in the kidneys of calves under three years old often stay within the permitted range, older cattle tend to collect more of this metal in locations with greater soil cadmium content, frequently above the allowable limit [129, 130]. Also, ovine grazing on natural pastures, usually raised in extensive grazing systems, are particularly exposed to cadmium; therefore, they can accumulate higher amounts of this metal than other ruminants and can be considered a good indicator of cadmium contamination in a given area [131–133]. A correlation between the sex and age of the bovine was also observed, as cadmium (Cd) levels in the liver and kidneys were found to be higher in female cattle older than four years [134]. Previous research has revealed that the kidneys, liver, heart, and intestines have the highest quantities of lead (Pb) and cadmium (Cd), but these heavy metals are not discovered in the lungs [135].

1.4. Preparation of protein hydrolysates from meat by-products

1.4.1. Lyophilisation process

Processing animal by-products in a way that preserves their nutritional value would contribute to reducing disposal costs and negative environmental impacts [136]. Recent research in Lithuania has shown that lyophilisation allows preserving the nutritional and bioactive properties of proteinaceous materials, making it a good technology for preserving nutrients [137]. Lyophilization is regarded as a high-quality preservation technology, as it effectively reduces moisture content and water activity, thereby slowing spoilage reactions and inhibiting microbial growth [136, 138–141]. Freezing water before lyophilization stops chemical, biochemical and microbiological processes, so this method allows the organoleptic characteristics and nutrient content of the products to be maintained unchanged [43]. Among the available methods, lyophilization is considered one of the most advanced, allowing long-term preservation while retaining most biologically active compounds compared with other drying processes [138, 139].

The lyophilization process can be effectively applied to the preservation of protein hydrolysates and bioactive peptides, ensuring their structural stability, retention of bioactive properties, and suitability for further use in functional food products [142, 143]. Also, studies show that this process has a negligible effect on protein and fat content, and also helps to preserve fat-soluble vitamins (A and D) and extends the shelf life of products up to two years [144, 145]. Lyophilisation does not significantly affect the fatty acid

composition and is therefore considered a suitable method to preserve their stability [64].

1.4.2. Enzymatic hydrolysis

Although underutilization of meat by-products remains a common issue within the red meat industry, technological advancements and improved processing and recovery systems present opportunities to enhance profitability while delivering positive environmental outcomes [146, 147]. Due to their high protein content, these by-products are well-suited for enzymatic hydrolysis, which releases bioactive peptide fractions with great potential for the development of functional foods and nutraceuticals aimed at preventing and managing chronic diseases [148–150]. Enzymatic hydrolysis is a well-researched and adaptable approach for improving the functional characteristics of animal-derived protein hydrolysates [151]. The application of this process in the pharmaceutical and nutraceutical industries is particularly appreciated due to the low content of toxic chemical compounds and organic solvents, as well as the high specificity and accuracy that allows control over the structure and composition of the final peptides [152]. During hydrolysis, proteins are broken down into smaller peptide chains consisting of 2–20 amino acids, called hydrolysates, which become a valuable source of amino acids [153]. This method is characterized by high efficiency and product quality, as it takes place under mild technological conditions and, unlike chemical processes, does not cause unwanted side reactions [154]. Due to these properties, products obtained by enzymatic hydrolysis are distinguished by excellent functional characteristics and high nutritional value, therefore, they are considered promising compounds in both the food and health industries [155].

Protein hydrolysate represents an optimal form of protein source, characterized by superior nutritional properties, including a well-balanced amino acid composition and high digestibility. Nevertheless, its inherent bitterness has limited its incorporation into human diets, thereby constraining its practical applicability [31]. These hydrolysates can function as flavour enhancers, functional components, or nutritional supplements in foods with low protein quality. They are particularly valuable in dietary applications for individuals who have difficulty digesting whole or intact proteins. Protein hydrolysates rich in low–molecular–weight peptides – especially dipeptides and tripeptides with minimal amounts of free amino acids – offer broad nutritional applications and possess significant dietary and therapeutic benefits [32].

Porcine liver protein hydrolysates exhibit distinct functional properties, including resistance to acids, alkalis, and heat, as well as excellent solubility and water-binding capacity. These features, combined with their bioactive potential, make them highly valuable compounds [156]. Likewise, animal hearts contain physiologically active components with hypolipidemic effects, making them a rich protein source well suited for enzymatic hydrolysis [157]. Animal-derived proteins are a valuable source of essential amino acids that are often absent in plant-based proteins. These include methylhistidine and hydroxymethyllysine, which can be utilized in the production of peptides with antioxidant properties [158]. Liver proteins are rich in aromatic (tyrosine, phenylalanine) and hydrophobic (leucine, valine, isoleucine) amino acids, which contribute to their strong antioxidant activity [159]. Meanwhile, heart tissues serve as a source of biologically active compounds with hypolipidemic effects [157]. Furthermore, the biological activity of protein hydrolysates obtained by this process can be improved, as it is influenced by several factors: the type of enzyme used, the duration of treatment, pH, and the temperature settings applied during the extraction process [160–163].

Depending on the substrate features and hydrolysis aims, a variety of commercial proteolytic enzymes are used, including trypsin, pepsin, papain, alkaline proteases, flavour proteases, and neutral proteases [164]. These enzymes, which originate from plants, animals, or microbes, exhibit various substrate specificities that influence the chemical composition and bioactivity of the resultant hydrolysates. For example, papain may break down peptide bonds in hydrophobic protein areas [165]. Unique proteases produce peptides with various amino acid sequences and functional characteristics, resulting in variances in antioxidant activity [166]. Various proteolytic enzymes with different catalytic properties are commonly used in protein hydrolysis and the production of bioactive peptides, including flavourzyme, pepsin, and papain [167].

Flavourzyme is a proteolytic enzyme complex extracted from *Aspergillus oryzae*, which has a unique mechanism of action combining endopeptidase and exopeptidase activity [168]. Due to this property, it not only cleaves peptide bonds, but also removes terminal amino acids, resulting in hydrolysates with reduced bitterness and improved sensory properties [169, 170]. Flavourzyme can also efficiently hydrolyze proteins of various origins and generate peptides with antioxidant and antihypertensive properties [171, 172].

Pepsin is an endopeptidase that cleaves peptide bonds in aromatic amino acids such as phenylalanine and tryptophan, and works best in an acidic environment at 37–42 °C [173]. Meanwhile, papain, also known as papaya protease I, is a cysteine protease obtained from the fruit of the papaya *(Carica*

papaya) [174]. This enzyme has a good level of thermal stability and cleaves peptide bonds behind arginine or lysine residues when preceded by hydrophobic amino acids, making it versatile in the hydrolysis of various protein substrates [173]. Porcine myofibrillar proteins hydrolyzed by papain have strong metal chelating and free radical scavenging properties [175]. Several studies have also highlighted pepsin as an effective enzyme for extracting antioxidant peptides.

1.5. Functional properties and applications of protein hydrolysates and bioactive peptides from meat by-products

Bioactive peptides constitute a significant portion of protein hydrolysates and are widely recognized for their potential in the development of functional food ingredients. These peptides are short chains of 2–20 amino acid residues released during enzymatic hydrolysis, which, although inactive in the natural protein structure, can have beneficial physiological effects on the human or animal body [57, 152, 176, 177].

Due to their specific amino acid composition, peptides and protein hydrolysates exhibit antioxidant and antibacterial properties that contribute to the inhibition of oxidative processes and microbial spoilage in food products [178, 179]. Studies have shown that bioactive peptides derived from food proteins can effectively reduce lipid oxidation, autoxidation and free radical formation, thus acting as natural and safe antioxidants with high bioavailability and efficacy [176, 180–182]. Meat by-products are a valuable and sustainable source of essential amino acids and bioactive peptide mixtures [18, 183]. Hydrolysates obtained from these raw materials can be used as functional food ingredients, providing both nutritional and biological value [184]. These compounds exhibit a wide range of beneficial effects, including antioxidant, anti-fatigue, anti-inflammatory, antihypertensive, antimicrobial, immunomodulatory, anticoagulant and opioid-like activities [12, 185]. As a result, they can affect many physiological systems, such as the cardiovascular, immune, nervous and digestive systems, and may contribute to the prevention and management of various diseases, including metabolic disorders, cancer and neurological diseases [22, 32].

Due to their composition, which is rich in vitamins, minerals and amino acids, meat by-product hydrolysates have great industrial potential, especially in the food sector [186]. They can be used as emulsifiers, water-binding agents and natural flavour enhancers, improving the technological and organoleptic properties of food products, as well as extending their shelf life [30].

In addition to food applications, these compounds are gaining increasing attention in the pharmaceutical field due to their cost-effective production,

low risk of undesirable side effects, and reduced potential for drug resistance [187, 188]. In addition, the inclusion of protein hydrolysates in animal feed can reduce oxidative stress, enhance immune function, and promote animal health under changing environmental conditions [189, 190].

1.6. Antioxidant and antimicrobial properties of protein hydrolysates and their mechanisms of action

Antioxidant peptides and protein hydrolysates are compounds that can slow down or prevent oxidative processes. Oxidation is initiated by free radicals, including reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), lipid peroxyl radical ($ROO^{\bullet}$), and reactive nitrogen species, such as nitric oxide ($NO^{\bullet}$) [191, 192]. Hydrophobic, acidic, and basic amino acid residues are commonly found in peptides and protein hydrolysates that exhibit antioxidant properties [193]. Peptides rich in hydrophobic amino acids can protect lipids from oxidation by forming a physical barrier within cell membranes. Due to their surface-active characteristics, these peptides can develop a protective film or coating at the oil–water interface, thereby preventing lipids from direct contact with oxidizing agents such as free radicals. In this way, they reduce intracellular oxidative processes and safeguard cell membranes from damage induced by peroxyl radicals [192].

It has usually been demonstrated that protein hydrolysates and peptide fractions with a high percentage of low-molecular-weight peptides have potent antioxidant activity [194]. Peptides and hydrolysates that are rich in sulfur-containing, nucleophilic amino acids like cysteine (Cys) and methionine (Met), as well as aromatic residues like phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr), or imidazole-ring amino acids like histidine (His), are generally linked to antioxidant potential. Furthermore, the location of amino acid sequences might influence antioxidant ability; for example, hydrophobic residues near the N-terminal end of a peptide, such as leucine (Leu) or valine (Val), may increase its bioactivity [195].

Peptides and protein hydrolysates with antibacterial properties play an important role in preventing the spread of pathogenic microorganisms and food spoilage [196]. The main types of microorganisms responsible for the spoilage of meat and meat by-products are *Pseudomonas* spp. And lactic acid bacteria (LAB) [197]. Meanwhile, *Salmonella Typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* are the pathogens most commonly associated with human foodborne illness [198]. As noted by other authors, porcine blood protein hydrolysates have shown antimicrobial activity against these and other microorganisms – *Listeria*

monocytogenes, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus* [199].

The mechanism of action of antimicrobial peptides is still not fully understood, although they are widely studied. Their antibacterial activity is determined by several main factors: electrostatic interactions between the positively charged peptide groups and the negatively charged lipids present in the membrane of microorganisms, as well as the hydrophobicity and flexibility of the peptide, which allow it to penetrate the membrane [200]. Food components such as proteins, proteases, fats and metal ions can interfere with antimicrobial peptides from interacting effectively with target pathogens [201, 202].

Antimicrobial peptides have garnered increasing attention from scientists in recent years due to their broad range of activity and are viewed as a viable tool in the fight against the spread of drug-resistant microbes. Antimicrobial peptides are an integral part of the innate immune system [19]. These short-chain peptides are able [194] to defend organisms against the invasion of harmful microorganisms. The mechanism of their bacteriostatic activity involves both non-specific interaction with membrane phospholipids and binding to specific receptors in the cell membrane. These peptides also exhibit a broad spectrum of antibacterial activity [203]. Due to these properties, antimicrobial peptides are considered a promising strategy for the development of new antibacterial drugs, which also indicates that they have a low potential to induce drug resistance [204]. A large number of bioactive peptides have been extracted from porcine by-products by enzymatic hydrolysis [205]. Other researchers have reported that hydrolysates obtained from enzymatically treated porcine by-products exhibited antibacterial activity at different time intervals, and several peptide sequences were identified, including GLNQALVDLHLGSAR, ALFQDVQKPSQDEWGK, LSGPQAGLGEYLFER, and LGEHNIDVLEGNEQFINAAK [159].

1.6.1. Sensory challenges and stability issues of protein hydrolysates

The primary challenge in using peptide mixtures is their instability, as they are susceptible to enzymatic degradation, pH fluctuations, and temperature changes during food processing and storage, which can lead to a decrease in their effectiveness over time. In addition, some peptides can impart undesirable sensory properties to food products, thus reducing their appeal to the consumer [193]. The formation of bitter taste in hydrolysates remains one of the main challenges in their practical application, as this undesirable sensory property is characteristic of most food protein hydrolysates and may limit their integration into food products and consumer acceptability

[206–208]. This bitterness is usually attributed to the presence of low molecular weight peptides [209]. Therefore, to ensure appropriate sensory properties of the final product, it is necessary to apply effective bitterness masking strategies and carefully optimize the hydrolysate concentration. Meat proteins are very diverse, and enzymatic hydrolysis can produce a wide range of peptides, some of which may have the ability to modify or mitigate the bitter taste, thus improving the organoleptic properties of the product [193, 210, 211].

Masking is one of the methods to eliminate the bitter taste of hydrolysates and improve the taste properties of the product [211]. The use of masking agents is considered one of the simplest and most effective ways to reduce the bitterness of enzymatic protein hydrolysates (EPH) in industry, as this method does not impair their nutritional value or biological activity [212]. These methods aim to reduce the solubility of bitter compounds, induce their interactions with taste receptors, and inhibit signals to the appropriate system, thereby reducing the sensation of bitterness [206, 213–215]. Gelatine, modified starch, and malic or citric acid can all be used to lessen the disagreeable taste of hydrolysates [216, 217]. Bertelsen et al. claim that xylitol, sucrose, α-cyclodextrin, and maltodextrin have been demonstrated to function as efficient taste masking agents, successfully lessening the bitterness of soy protein hydrolysates [215]. Also, scientific studies have shown that xylitol, sucrose and maltodextrin significantly reduce bitterness in aqueous solutions [211].

1.6.2. Application of protein hydrolysates in functional food systems

The application of protein hydrolysates in food products is considered a promising direction, and their use includes human nutrition and animal feed production [218]. Protein hydrolysates are widely used in human nutrition as functional ingredients due to their good digestibility and high bioavailability [184]. In the pet food industry, protein hydrolysates are widely used in product formulation to reduce the risk of allergic reactions and improve the sensory properties and acceptability of the feed [219]. These compounds are considered promising nutraceuticals with significant potential in the field of animal and people health promotion [218].

As the prevalence of chronic diseases such as cardiovascular disorders, diabetes, and obesity continues to rise, consumers are increasingly seeking functional foods that combine nutritional value with health benefits [220, 221]. In this context, consumers are increasingly looking for functional foods and food supplements that fit their lifestyles and provide overall sensory,

nutritional and health-promoting effects. Peptides, with their unique sensory properties and diverse biological activities, are attracting increasing scientific and industrial interest [220].

Gummy candies are among the most popular confectionery products enjoyed by consumers of all ages, from children to seniors, even though the main component of these products is sugar [222–224]. The use of gummies as a method of delivering active chemicals is one of the current areas in drug and functional ingredients delivery research. Because of their pleasant flavour, texture, and convenience of use, gummies are becoming more and more popular as a means to give vitamin and mineral supplements [225, 226]. Gummy supplements are easy to consume, suitable for people of all ages who can chew easily, are non-invasive, and have an attractive appearance and colour [227–229]. However, gummy forms also have certain disadvantages – they often have a high sugar content, require chewing, and there are also difficulties in masking unwanted tastes [230, 231].

Eating them may raise your chance of developing chronic conditions, including type 2 diabetes, obesity, dental caries, and hyperglycemia [232]. To improve the nutritional value of gummy candies and jellies, traditional sugar can be replaced with natural sweeteners or by-product components [233, 234]. Combining sustainability and practicality, the use of hydrolysates in confectionery products has the potential to change the way the industry selects ingredients. Synthetic preservatives such as BHA and BHT are commonly used in conventional confectionery, raising concerns about consumer safety [235]. Natural antioxidants and antibacterial compounds derived from liver protein hydrolysates could be a sustainable and consumer-friendly alternative to these chemicals, helping to eliminate artificial additives, extend product shelf life, and meet clean label requirements [236].

2. RESEARCH METHODS AND MATERIALS

The research was conducted at the Department of Food Safety and Quality, Faculty of Veterinary Medicine, Veterinary Academy, Lithuanian University of Health Sciences, during the period of 2021–2025. All samples required for the study were collected from two slaughterhouses in Lithuania.

2.1. Ethical statement

This study did not require authorisation or ethical approval under Directive 2010/63/EU of the European Parliament and of the Council, as live animals were not used for experimental purposes and were not specifically killed for the study. All samples (liver, heart, lung, kidney) were collected post-mortem from animals slaughtered during commercial food production. The animals were slaughtered in accordance with standard industry practice under the supervision of an official veterinary service. All animal-related procedures were carried out in accordance with the requirements of European Union Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing (Official Journal of the European Union L 303/1). The animal by-products used in the study were handled and in accordance with Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down provisions on the handling and health protection of animal by-products and derived products. The biological samples collected were handled in accordance with current biosafety and laboratory testing standards. Sample collection, transportation, and storage were carried out in a manner that ensured their suitability for analysis and avoided potential contamination.

2.2. Design of the study

This dissertation focused on the safety and quality assessment of animal meat by-products and their application in the production of functional food products. The overall research design and sequence of experimental procedures are presented in Fig. 2.2.1. The study was conducted in several stages, including the assessment of the safety, nutritional value and suitability for processing of animal by-products (porcine, bovine and ovine); enzymatic hydrolysis of selected raw materials and extraction of peptide hydrolysates; evaluation of the antioxidant, antibacterial and functional properties of the resulting hydrolysates; and the development and evaluation of functional food prototypes.

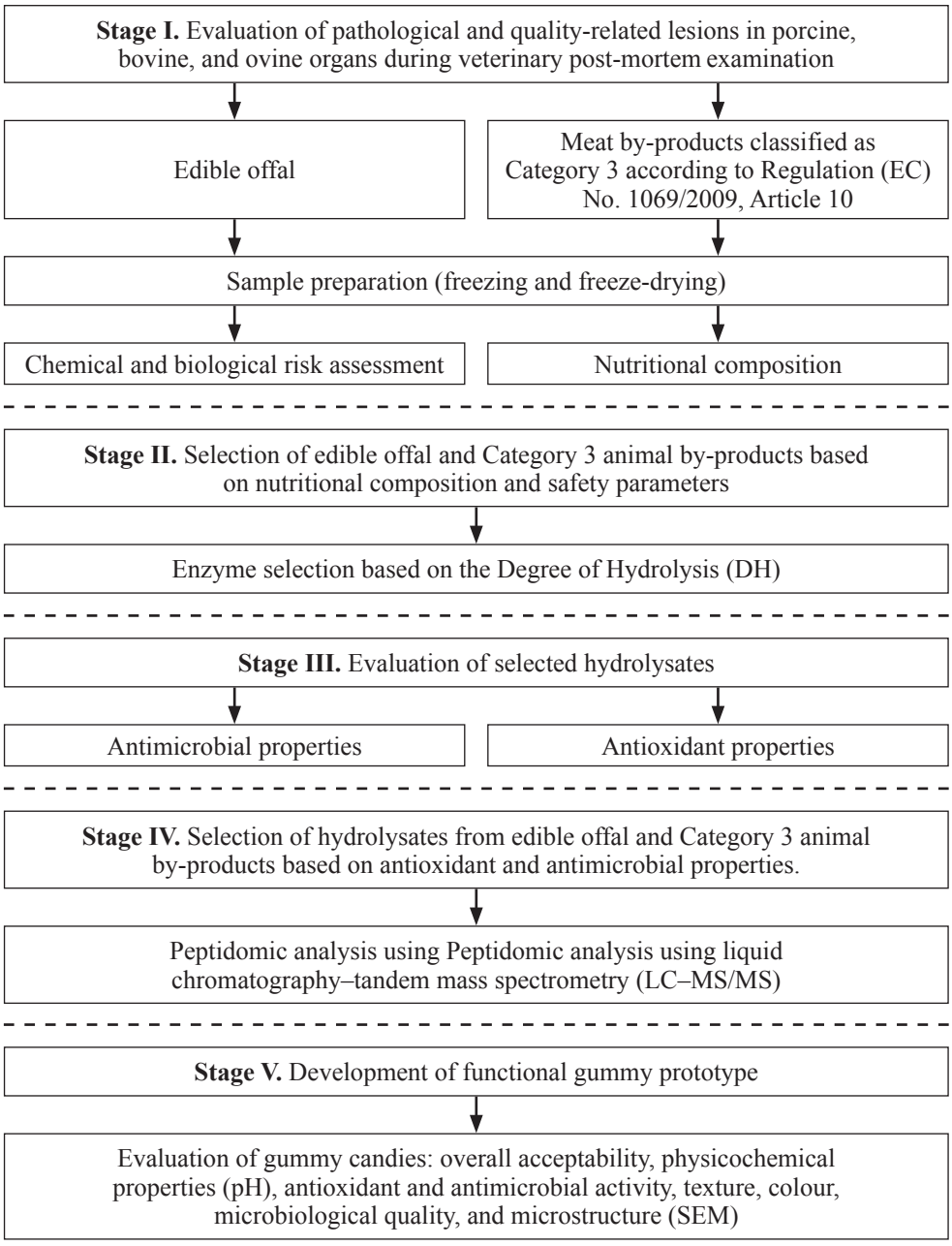


Fig. 2.2.1. *Study scheme*

2.3. Methodology of post-mortem organ inspection

Post-mortem lesions in organs were evaluated during veterinary post-mortem inspections carried out at slaughterhouses X and Y. A total of 600 organ samples (liver, heart, kidney, and lung) obtained from porcine, bovine, and ovine animals were examined, while ovine samples were collected from slaughterhouse Y. Within the bovine group (n = 600), cows accounted for 54.5 % (n = 327), bulls for 27.0% (n = 162), and heifers for 18.5% (n = 111).

Veterinary post-mortem inspection was performed in accordance with the official veterinary control methodology and the provisions of Commission Implementing Regulation (EU) 2019/627 [237]. During the post-mortem inspection, a systematic assessment of the edibility of the carcass and offal was carried out, using standardized examination methods: visual inspection, palpation and, if indicated, tissue sections.

Decisions on the use of offal were made based on the provisions of the quality system operating instructions KT–2–3–1–D1 "Edibility Assessment of Meat and Other Slaughter Products in Farm Animal Slaughterhouses" approved by the Director of the State Food and Veterinary Service by Order No. B1-270 of 22 April 2020 [238].

The assignment of animal by-product category to the appropriate categories was carried out in accordance with the requirements of Regulation (EC) No 1069/2009 [239].

The detected changes in by-products were systematically recorded and classified, taking into account their nature and assigning them to the appropriate categories of animal by-products (Table 2.3.1).

Table 2.3.1. *Classification of pathological changes according to animal by-product categories*

Organ	Category 2 animal by-products	Category 3 animal by-products
Liver	Hepatic abscesses; hepatic fasciolosis; chronic cholangitis; focal hepatic necrosis	Hepatic fatty degeneration; hepatic hyperaemia; hepatic fibrosis
Heart	Fibrinous pericarditis; endocarditis	Myocardial hemorrhages
Lung	Pneumonia; pulmonary aspiration	Pulmonary emphysema
Kidney	Chronic interstitial nephritis; nephritis	Renal cysts

2.4. Sample preparation of edible offal and Category 3 animal by-products

Meat by-products exhibiting pathological lesions were collected and classified as Category 3 animal by-products in accordance with Regulation (EC) No 1069/2009. These included liver samples (n = 8 × 3) with hepatic fatty degeneration; hepatic hyperaemia; hepatic fibrosis, heart samples

(n = 8 × 3) with myocardial hemorrhages, lung samples (n = 8 × 3) with pulmonary emphysema and kidney samples (n = 8 × 3) with renal cysts. The organs were obtained from porcine, bovine, and ovine animals that were deemed fit for slaughter following veterinary ante-mortem inspection.

Samples that showed no pathological changes at post-mortem examination were selected and considered edible offal (fit for human consumption). The control group consisted of liver, kidney, heart and lung samples from porcine, ovine and bovine (n = 8 × 3). The samples were transported to the laboratory within 24 hours after slaughter and stored in sealed containers at 4 °C until further analysis.

Before the freeze-drying procedure, Category 3 meat by-products and edible offal were cut into 2 × 2 cm pieces.

2.4.1. Freeze-drying

Edible offal and Category 3 meat by-products, as defined in Article 10(4) of Regulation (EC) No. 1069/2009 were rapidly frozen at −35 °C for 3 h using a Liebherr freezer (LGv 5010 MediLine) and subsequently freeze-dried in a lyophilizer (Harvest Right, North Salt Lake, UT, USA) at −80 °C under a vacuum pressure of 20 Pa for 72 h. The freeze-dried samples were then milled using a laboratory-scale mill (Fritsch Pulverisette 14, Idar-Oberstein, Germany) operating at 6000 rpm and sieved through a 200 µm mesh.

2.5. Determination of the nutritional composition of edible offal and Category 3 animal by-products

2.5.1. Protein and fat content

The protein content was estimated by the Kjeldahl method according to the standard LST ISO 937:2023 “Meat and meat products: Nitrogen content” [240]. Fat content was analysed using the LST ISO 1443:2000 [241] standard method for the determination of total fat in meat and meat products. The moisture content was determined according to the standard LST ISO 1442:2023 “Meat and meat products: Determination of moisture content (reference method)” [242].

2.5.2. Determination of fatty acid composition

The fatty acid composition was determined using standardised analytical procedures. Samples preparation was carried out according to LST EN ISO 12966-2:2017, Part 2: Preparation of methyl esters of fatty acids [243], and chromatographic analysis followed ISO 12966-1:2014 [244]. Fatty acid methyl esters (FAMEs) were analysed with a PerkinElmer® Clarus 680 gas

chromatograph coupled to a PerkinElmer® Clarus SQ8T mass spectrometer (PerkinElmer, Inc., Norwalk, CT, USA), column – SP-2560, length – 100 m, diameter – 0,25 mm, 0,2 μm (Sigma-Aldrich). The chromatographic column temperature program was as follows: initial temperature of 60 °C (held for 1 min), increased at 12 °C/min to 250 °C, and maintained for 10 min. The spectrometer temperature program was 5 °C/min up to 300 °C, with a 20 min hold. The injector temperature was set at 250 °C. A 1 μL aliquot of the prepared sample from the upper separated methyl ester phase was injected into the injector. Quantification was performed using a calibration curve prepared with the Supelco 37 Component FAME Mix standard (Merck & Co., Darmstadt, Germany).

The resulting fatty acids were classified into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). PUFAs were further divided into n-6 and n-3 subgroups based on the position of the first double bond. Unsaturated fatty acids (UPFA) were calculated as the sum of MUFA and PUFA.

2.5.3. Calculation of lipid indices

The health lipid indices were evaluated by summing the saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFA) to identify the essential fatty acids (EFA) [245]. The atherogenic index (AI) and thrombogenic index (TI) were derived using the Ulbricht & Southgate [246]. The hypocholesterolemic to hypercholesterolemic ratio (h/H) was obtained following the method of Fernandez et al. [247].

$$AI = \frac{C12{:}0 + 4 \cdot C14{:}0 + C16{:}0}{\Sigma MUFA + \Sigma PUFA}$$

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0.5 \cdot \Sigma MUFA + 0.5 \cdot \Sigma(n-6PUFA) + 3 \cdot \Sigma(n-3PUFA) + \left(\frac{\Sigma(n-3PUFA)}{\Sigma(n-6PUFA)}\right)}$$

$$h/H = \frac{C18{:}1 + \Sigma PUFA}{14{:}0 + 16{:}0}$$

2.5.4. Determination of amino acid composition

The hydrolysis of amino acids from defatted samples was carried out in accordance with Commission Regulation No. 152/2009 [248]. After hydrolysis, the materials were cooled to room temperature before being further processed. Then, 1 mL of filtered hydrolysate was pipetted into a 100 mL

volumetric flask, along with 50 mL of 100 mmol/mL L-2-aminobutyric acid used as an internal standard. The contents of the flask were diluted to the mark with ultrapure water, properly mixed, and filtered using a 0.22 mm syringe filter.

Derivatisation reagents, calibration standards, and sample preparation were performed according to the Waters AccQ Tag chemistry kit instruction manual [249]. Amino acid analysis was performed using a Shimadzu low-pressure gradient HPLC system (Shimadzu Corp., Kyoto, Japan) equipped with an LC-10ATVP solvent delivery module, SIL-10ADVP autoinjector, CTO-10ACVP column oven, RF-10AXL spectrofluorometric detector, SCL-10AVP system controller, and DGU-14A online degasser. System control and data acquisition were controlled using LabSolution LC software (Shimadzu Corp., Kyoto, Japan). Amino acid derivatives were separated on a Nova-Pak C18 chromatography column (150 × 3.9 mm, 4 μm; Waters Corp., Milford, MA, USA) maintained at 37 °C with an injection volume of 10 μL. Detection was performed using fluorescence with excitation at 250 nm and emission at 395 nm. Gradient elution was applied at a flow rate of 1.0 mL/min using eluent A (100 mL of Waters AccQ Tag eluent A concentrate diluted in 1 L of ultrapure water), eluent B (acetonitrile), and eluent C (ultrapure water).

To effectively separate amino acid molecules, we employed the gradient algorithm that follows: eluent A was reduced from 100% to 98% and eluent B was raised from 0% to 2% over 0.0–0.5 min; A was reduced from 98% to 94% and B raised from 2% to 6% over 0.5–18.0 min; A was reduced from 94% to 90% and B raised from 6% to 10% over 18.0–19.0 min; A was reduced from 90% to 83% and B raised from 10% to 17% over 19.0–29.5 min. Subsequently, A was reduced from 83% to 0%, while B was raised from 17% to 60% and C from 0% to 40% over 29.5–35.0 min. The mobile phase was then held at 60% B and 40% C for 5.0 min, followed by a linear increase of A from 0% to 100% within 1.0 min, and finally maintained at 100% A for 10.0 min. By contrasting the retention periods and peak regions of the separated sample components with those of the standard solution, specific amino acids were identified and quantified.

2.6. Assessment of biological and chemical safety of edible offal and Category 3 animal by-products

2.6.1. Microbiological analysis

The total aerobic plate count was determined on Plate count agar (PCA, Liofilchem, Italy) after incubation at 30 °C for 72 h, in accordance with EN ISO 4833-1:2013 [250]. *Enterobacteriaceae* were enumerated on Violet Red

Bile Glucose Agar (VRBG, Liofilchem, Italy) after incubation at 37 °C for 24 h and confirmed by tests for glucose fermentation and oxidase activity, according to EN ISO 21528-2:2017 [251].

The enumeration of *Escherichia coli* was performed on Tryptone Bile X-glucuronide Agar (TBX, Biolife, Monza, Italy) after incubation at 44 °C for 24 h, in accordance with ISO 16649-2:2001 [252]. The detection of *Listeria monocytogenes* (25 g) was carried out on *Listeria* agar according to Ottaviani and Agosti (ALOA, Liofilchem, Italy) following EN ISO 11290-1:2017 [253].

Salmonella spp. Detection (25 g) was performed according to ISO 6579-1:2017, using selective plating on Xylose Lysine Deoxycholate Agar (XLD, Liofilchem, Italy) and Brilliant Green Agar (BGA, EO Labs, Scotland, UK).

2.6.2. Determination of pesticide residues

Each sample was ground to a fine powder, and 5.0 ± 0.1 g was weighed into a 50 mL polypropylene centrifuge tube. Then, 220 µL of the internal standard (TPP) solution and 7.8 mL of acetonitrile were added, bringing the total volume to 10 mL. A salt mixture was added, the sample was shaken vigorously for 1 minute, and then centrifuged for 5 minutes at ≥ 3000 g.

The upper acetonitrile phase (approximately 7 mL) was transferred to a 12 mL PP tube and stored in a freezer (≤ –18 °C) for at least 2 hours. Subsequently, 5 mL of the cold extract was transferred to a new 15 mL PP tube containing 120 mg PSA and 850 mg magnesium sulfate, shaken vigorously for 45 seconds, and centrifuged for 5 minutes at ≥ 3000 g.

The resulting acetonitrile phase was transferred into a dark glass chromatographic vial for analysis.

The analysis was performed using an LC–MS/MS (ESI–MS/MS) system equipped with a Waters ATLANTIS dC18 (2.1 × 100 mm, 3 µm) column. The column temperature was maintained at 40 °C, the flow rate was 0.25 mL/min, and the injection volume was 10 µL. The total chromatogram run time was 30 minutes, applying a gradient elution mode as follows: at 0 min – 100% of phase 7.5.2 and 0% of phase 7.5.1; at 15 min – 0% of phase 7.5.2 and 100% of phase 7.5.1; this composition was maintained until 25 min; at 26 min – 100% of phase 7.5.2 and 0% of phase 7.5.1; and at 30 min – 100% of phase 7.5.2 and 0% of phase 7.5.1.

Analytical data were processed using the MassLynx software package. The pesticide concentration in the sample was calculated using the formula:

$$C = \frac{C_T \cdot V_{ACN}}{m_{sample}}$$

where: C – pesticide concentration in the sample, mg/kg; C_T – analyte concentration in the extract (μg/mL), V_{ACN} – volume of acetonitrile (mL), m_{sample} – sample mass (g).

2.6.3. Determination of heavy metal content

The lyophilized by-product samples were ground in a laboratory mill to obtain a homogeneous mass (particle size ≤ 0.5 mm). During the grinding, care was taken to avoid overheating and possible contamination. The prepared samples were stored in sealed containers until analysis.

From each homogenized sample, 0.50 ± 0.01 g was weighed into acid-resistant mineralization vessels using an analytical balance.

Samples for heavy metals (Hg, Cd and Pb) were mineralized by adding 5 mL of concentrated nitric acid (HNO_3, 65–70%) and 1 mL of hydrogen peroxide (H_2O_2, 30%). Mineralization was performed under pressure, increasing the temperature to 180–210 °C and maintaining it until the organic matrix was completely decomposed.

After mineralization, the samples were cooled and filtered. The resulting solutions were transferred to volumetric flasks and diluted with deionized water to a final volume of 25 mL.

Elemental analysis was performed using an ELAN DRC II inductively coupled plasma mass spectrometer (ICP-MS; PerkinElmer/SCIEX, USA).

2.7. Preparation of animal by-product hydrolysates

Lyophilized porcine, ovine, and bovine livers and hearts were ground to a fine powder using a Fritsch Grind Pulverisette 14 laboratory mill, and subsequently, they were sieved through 200 μm sieve. Enzymatic hydrolysis was carried out using papain (≥ 30,000 USP-U/mg), pepsin (≥ 250 U/mg), and Flavourzyme® (≥ 500 LAPU/g) obtained from Sigma-Aldrich (St. Louis, MO, USA). For sample preparation, the lyophilized samples were mixed with ice in a 1:1 ratio. Optimal conditions for each enzyme were determined as follows: papain (37 °C, pH 6) pepsin (37 °C, pH 2.5), flavourenzyme® (50 °C, pH 5.5). The pH of the hydrolysates was adjusted using 1 N NaOH or 1 N HCl. The enzyme-to-substrate ratio used during all incubation periods was 10 g/kg (m/m). Incubation times were 3, 6, and 24 hours. After incubation, the hydrolysates were heated to 95 °C to inactivate the enzymes. They

were then cooled in an ice bath. The hydrolysates were filtered through filter paper and centrifuged at 4000 rpm for 10 minutes at 4 °C. The supernatant of each hydrolysate was frozen at −80 °C until further analysis.

2.8. Determination of degree of hydrolysis (DH) following enzymatic treatment

Hydrolysates of porcine, ovine, and bovine liver and heart were evaluated by determining the proportion of TCA-soluble peptides. The degree of hydrolysis was estimated as the percentage of 10% (w/v) trichloroacetic acid (TCA)-soluble compounds relative to the total protein content of the sample. Protein precipitation was performed by mixing 500 µL of the hydrolysate with an equal volume of 20% (w/v) TCA, resulting in a final TCA concentration of 10% (w/v) [254]. The mixture was incubated for 30 min at room temperature and subsequently centrifuged at 3500 rpm for 15 min. The supernatant was appropriately diluted, and the protein content of the TCA-soluble fraction was determined according to the method of Hartree [255]. Results were expressed as milligrams of protein using bovine serum albumin as the standard.

$$\text{DH (\%)} = \left[\frac{\text{Solubilized protein content in 10\% TCA (mg)}}{\text{Total protein content (mg)}}\right] \cdot 100$$

2.9. Determination of antioxidant activity of hydrolysates

2.9.1. DPPH• free radical scavenging assay

The ability of the hydrolysate to remove radicals (DPPH•) was tested following the method described by Brand-Williams et al. [256], with a slight modification regarding the amount of antioxidant solution used [257]. A volume of 0.04 mL of the sample was mixed with 0.96 mL of a 6 µM DPPH• solution in 75% methanol. Absorbance was measured three minutes after the reaction started at a wavelength of 515 nm using a J.P. ELECTA S.A. V-1100D spectrophotometer from Barcelona, Spain. A solution of 75% methanol in distilled water was used as the blank. The scavenging effect was calculated using the following equation.

$$\text{Scavenging activity (\%)} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}}\right)\right] \cdot 100$$

2.9.2. ATBS$^{\bullet+}$ radical-cation scavenging assay

The ATBS$^{\bullet+}$ radical cation was made following the method described in [258]. It was created by mixing a 7 mM aqueous solution of ABTS$^{\bullet+}$ in absolute ethanol with 2.45 mM potassium persulfate (weight by weight). The mixture was stored in the dark at room temperature for 12 to 16 hours. Before using it, the ABTS$^{\bullet+}$ solution was diluted with absolute ethanol to get an absorbance of 0.8 ± 0.03 at a wavelength of 734 nm. A reagent blank was also measured as a control. Then, 20 µL of meat by-product hydrolysate at various concentrations was added to 980 µL of the ABTS solution and let react for 6 minutes. The resulting mixture was analyzed using a J.P. SELECTA S.A. V-1100D spectrophotometer (Barcelona, Spain). The scavenging effect was calculated using the following equation.

$$\text{Scavenging activity (\%)} = \left[1 - \left(\frac{A_{sample}}{A_{control}}\right)\right] \cdot 100$$

2.10. Determination of antimicrobial activity of hydrolysates

2.10.1. Antibacterial activity of hydrolysates using the agar well diffusion method

The agar well diffusion method was used to evaluate the antibacterial activity of hydrolysates derived from porcine, bovine, and ovine meat by-products. Reference strains, including *Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* subsp. *Aureus* ATCC 25923, *Salmonella enterica* subsp. *Enterica* serovar Typhimurium ATCC 14028, and Escherichia coli ATCC 25922, were pre-cultivated on PCA (Plate Count Agar, Liofilchem, Roseto degli Abruzzi, Teramo, Italy) slants at 37 °C or 30 °C overnight. Mature cultures were suspended in sterile physiological saline (0.85% NaCl in distilled water), and cell density was adjusted to McFarland standard No. 0.5. One milliliter of each standardized suspension was added to 100 mL of melted PCA medium cooled to 45 °C, thoroughly mixed, and poured into Petri dishes (90 mm, 12 mL per plate). After solidification, wells (8 mm in diameter) were cut into the agar and filled with 50 µL of the test solutions. Plates were incubated for 24 h at 37 °C or 30 °C, and antibacterial activity was determined by measuring inhibition zone diameters using callipers with 0.5 mm accuracy. Distilled water served as the negative control.

2.10.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of lyophilised and hydrolyzed meat by-products, in combination with papain, was determined using the broth microdilution method. Test samples were incorporated into Tryptic Soy Broth (Liofilchem Diagnostics, Roseto degli Abruzzi, Italy) to yield an initial concentration of 10 µg/mL, followed by twofold serial dilutions to obtain final concentrations of 5.0, 2.5, 1.25, 0.0625, 0.0313, 0.0156, 0.0078, and 0.0039 µg/mL. Each dilution was inoculated with 50 µL of a *Listeria monocytogenes* culture suspension (~1.5×10^8 CFU/mL). Controls consisted of bacterial suspensions in Tryptic Soy Broth without hydrolysates. All tubes were incubated at 37 ± 1 °C for 24 h. The MIC was defined as the lowest concentration of the test sample that inhibited visible bacterial growth upon visual inspection and was expressed in µg/mL. Experiments were performed in triplicate. Due to negligible inhibition zones, MICs of hydrolysates (after 24 h of papain hydrolysis) against *Salmonella enterica* subsp. *Enterica* serovar Typhimurium was not determined.

2.11. Peptidomic characterisation of hydrolysed porcine liver using LC–MS/MS

A 3 µL peptide mixture was loaded onto a reversed-phase trap column (PST C18, 5 µm particle size, 100 Å pore size, 180 µm × 20 mm; Waters Corporation, Wilmslow, UK) at a flow rate of 15 µL/min using 0.1% formic acid as the loading buffer. Peptides were subsequently separated on an analytical HSS-T3 C18 column (1.8 µm particle size, 75 µm × 250 mm; Waters Corporation, UK) with a linear gradient of 3–40% solvent B in A over 90 min. Solvent A was 0.1% formic acid, and solvent B was 100% acetonitrile with 0.1% formic acid. The separation flow rate was 300 nL/min, and the column temperature was maintained at 40 °C. Data acquisition was performed on a Synapt G2 mass spectrometer (Waters Corporation, Wilmslow, UK) coupled with MassLynx 4.1 software (Waters Corporation, Wilmslow, UK) in positive ion mode using a data-dependent acquisition (DDA) strategy. MS scans (0.6 s, m/z 350–1350) were followed by MS/MS analysis of the top seven precursor ions (charge states 2+, 3+, or 4+). MS/MS scans were acquired over a range of m/z 50–2000 with a duration of 0.6 s. After two MS/MS scans, precursor ions were dynamically excluded for 100 s, with a mass tolerance of 50 mDa. Label-free quantification and peptide identification were performed using Progenesis QI for Proteomics v4.2 (Nonlinear Dynamics) in combination with the MASCOT search engine v2.2.07. Raw files were imported into Progenesis under default parameters, and MS2 spectra were exported in mgf format for MASCOT analysis. Database

searches were carried out against the Sus scrofa reference proteome (Uniprot UP000008227, March 21, 2023) using the following parameters: no enzyme specificity, precursor mass tolerance of 20 ppm, and fragment mass tolerance of 0.3 Da. Search results were re-imported into Progenesis and mapped to MS1 features for quantification.

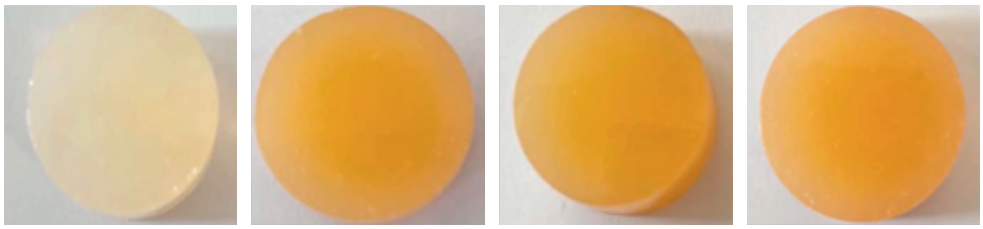
2.12. Preparation of gummy candy with porcine liver hydrolysates

Throughout the investigation, four distinct gummy formulations were created (Table 2.12.1). Agar powder, extracted from the algae *Gelidium sesquipedale*, was purchased from Rotmanka in Gdansk (Poland). Xylitol (Natur Hurtig Nuremberg, Germany) or sugar from Sanitex (Kaunas, Lithuania) was used to sweeten the candies. Citric acid was purchased from a local store, UAB Maxima (Kaunas, Lithuania). Gummy candies were made by adding 5 grams of hydrolysate. To mask the bitter taste of the hydrolysate, a liquid lime flavor, produced by Stockmeier Food GmbH (Herford, Germany), was added to the water. First, the agar powder was dissolved by heating it in water at 100 °C for 3 minutes. Xylitol was added to the mixture while boiling, and the mixture was continued to heat with constant stirring. Citric acid, hydrolysates, and flavouring were then added to the candy mixture. The mixture was poured into round silicone molds (1.9 cm in diameter and 1.5 cm in height). Each gummy weighed 2.05 ± 0.18 grams before drying and 2.01 ± 0.12 grams after drying.

Table 2.12.1. *Composition of gummy candies formulations containing porcine liver hydrolysates*

Gummy candies	Ag (g)	Water (mL)	Citric acid (g)	Xylitol (g)	Extract lime (mL)	Hydrolysate (g)
GC	10	100	1	10	0.1	
GC5Lpa3Ag	10	100	1	10	0.1	5
GC5Lpa6Ag	10	100	1	10	0.1	5
GC5Lpa24Ag	10	100	1	10	0.1	5

GC – gummy candies; 5.0 – content of hydrolysates (g); 3, 6, and 24 h – hydrolysis time; Pa – papain hydrolysis; Ag – agar.



GC GC5Pa3Ag GC5Pa6Ag GC5Pa24Ag

Fig. 2.12.1. *Visual representation of gummies supplemented with porcine liver hydrolysates*

GC – gummy candies; 5.0 – content of hydrolysates (g); 3, 6, and 24 h – hydrolysis time; Pa – papain hydrolysis; Ag – agar.

2.13. Microbiological profile of gummy candies with porcine hydrolysates

The microbiological assessment of gummy candy samples included the determination of total aerobic mesophilic bacteria, enterobacteria, and yeast and mould counts. Microbiological tests were performed on days 1, 7, and 21 of storage. Gummies were stored in hermetically sealed polyethene bags at 18–23 °C and 60–65% relative humidity, protected from direct sunlight. Sample preparation and serial decimal dilutions of gummy candy samples were performed in sterile, buffered peptone water, according to ISO 6887-1:2017 [266] and ISO 6887-4:2017 [267], which define general and product-specific procedures for processing confectionery samples. From each dilution, 1 mL was diluted twice for microbial enumeration. The total number of aerobic mesophilic bacteria was determined according to the ISO 4833-1:2013 [268] standard using plate count agar (PCA; Merck, Germany) after incubation at 30 ± 1 °C for 72 ± 3 h. Enterobacteriaceae were determined according to the ISO 21528-2:2017 [269] standard on violet red bile glucose agar (VRBG; Biolife, Italy) after incubation at 37 ± 1 °C for 24 ± 2 h. Yeasts and moulds were evaluated according to ISO 21527-1:2008 [270] using dichloran rose engal chloramphenicol agar (DRBC; Liofilchem, Roseto degli Abruzzi, Italy), incubated at 25 ± 1 °C for 5 days. Results are expressed as colony-forming units per gram of sample (CFU/g).

2.14. Nutritional composition of gummy candies

The composition of gummy candies was determined using standardized methods commonly applied in food analysis. Protein content was determined by the Dumas method (AOAC 990.03) [259]. Lipid content was determined using acid hydrolysis followed by solvent extraction according to AOAC 991.36 [240]. Moisture content was determined according to AOAC 925.45

[260] by drying approximately 3 g of ground sample until constant weight. Ash content was determined according to AOAC 923.03 [260]. Carbohydrate content was calculated by difference, subtracting the sum of protein, fat, moisture, and ash from the total mass.

2.15. Evaluation of physicochemical properties of gummy candies with porcine liver hydrolysates

2.15.1. Color coordinates

A portable colourimeter with CIE L*a*b*C*H* colour space selections, the PCE-CSM5 (PCE Instruments, UK), was used to evaluate the colour attributes. Lightness is indicated by L* in this scheme, the green-red colour axis by a*, and the blue-yellow colour axis by b*. The colour saturation is indicated by the C* (chroma) value, while the main spectral component (red, green, or blue) is defined by the H* (hue angle), which has values between 0° and 360°.

2.15.2. Texture

The texture (hardness) was assessed via a Brookfield CT-3 Texture Analyser (Middleboro, MA, USA).

2.15.3. pH

The pH of the samples was determined according to EN ISO 2917:2002 [261]. A pH meter (Inolab 3, Hanna Instruments, Milan, Italy) was used for the measurements. Before the analysis, the instrument was calibrated using standard buffer solutions with pH values of 4.01 and 7.00 (Sigma–Aldrich, St. Louis, MO, USA). After calibration, the pH electrode was inserted directly into the hydrolyzed chewy candy samples.

2.16. Overall acceptability of gummy candy with porcine liver hydrolysates

Using the ISO 8586-1 sensory evaluation approach, eight trained panel members evaluated the produced gummies containing porcine liver hydrolysates for overall acceptability [262].

2.17. Antioxidant activity in gummy candies

2.17.1. DPPH$^{\bullet}$ free radical scavenging assay

A standard DPPH radical solution was prepared by accurately weighing 0.0024 g of DPPH (±0.0001 g) and dissolving it in 80% (v/v) ethanol using an ultrasonic bath. The solution was then diluted to a final volume of 100 mL in a volumetric flask. Its absorbance was measured at 515 nm, with 80% ethanol serving as the reference. Samples of gummy candy extract were prepared by dissolving 1 g of the product in 100 mL of distilled water, stirring constantly for 1 hour at room temperature. After that, the mixture was centrifuged for 10 minutes at 4 °C at 8000 rpm. After collection and filtration through a 0.45 μm PTFE membrane filter, the supernatant was stored at −80 °C until further analysis.

The antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging assay, with each extract tested in triplicate. Antiradical activity was determined by mixing 60 μL of extract with 3 mL of a 6×10^{-5} ethanolic DPPH$^{\bullet}$ solution in a 1 cm cuvette and calculated from the proportion of stable DPPH$^{\bullet}$ radicals neutralised by antioxidant compounds in the sample. The decrease in absorbance at 515 nm was monitored spectrophotometrically until equilibrium was reached (approximately 30 min). Results were expressed as the percentage of DPPH$^{\bullet}$ radicals inhibited.

$$\text{Scavenging activity (DPPH}^{\bullet}) = \left(\frac{\text{Ab} - \text{Aa}}{\text{Ab}}\right) \cdot 100\%$$

where: Ab – absorbance of the blank sample (t = 0 min); Aa – absorbance of the sample with the tested chewing candy extract (t = 30 min).

2.17.2. ATBS$^{\bullet+}$ radical-cation scavenging assay

The ability to scavenge free radicals was assessed by the use of spectrophotometry. After making a 2 mM stock ABTS$^{\bullet+}$ solution, it was left to stand for 16 hours in a dark glass bottle. In order to get an absorbance of 0.800 at 734 nm, the stock solution was diluted with distilled water to create the working ABTS$^{\bullet+}$ solution. The reference solution, or blank, was distilled water. Samples of gummy candy extract were made by dissolving 1 g of the product in 100 millilitres of distilled water while stirring constantly for one hour at room temperature. After that, the mixture was centrifuged for 10 minutes at 4 °C at 8000 rpm. After being collected and passed through a

0.45 μm PTFE membrane filter, the supernatant was stored at –80 °C until further analysis was required.

$$\text{Scavenging activity } (ABTS^{\bullet+}) = \left(\frac{C \cdot V}{m}\right)$$

where: C – Trolox concentration (μmol/L), determined from a calibration curve; V – extract volume (L); m – accurately weighed mass of the sample (g).

2.18. Determination of the antimicrobial activity of gummy candies

The antimicrobial activity of the gummy candies was assessed using reference strains of food-associated conditionally pathogenic bacteria: *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* subsp. *Aureus* ATCC 25923, and *Salmonella enterica* subsp. *Enterica* serovar Typhimurium ATCC 14028. Petri dishes were prepared using the agar well diffusion technique. Once the agar solidified, the test gummies (10 × 10 mm) were placed directly on the agar surface. The plates were then incubated for 18–24 h at 30 °C for *B. cereus* and 37 °C for the remaining bacterial strains. After incubation, the inhibition zones (clear halos surrounding the gummies) were measured in millimeters. Gummies containing citric acid and agar served as the positive control, while sterile water with agar was used as the negative control. All experiments were conducted in triplicate.

2.19. Microstructural evaluation of gummy candies with porcine hydrolysates using SEM

Cross-sections of gummy candy samples were examined using a Mira3 scanning electron microscope (SEM) from Tescan Orsay Holding, a.s. (Brno-Kohoutovice, Czech Republic). The samples were manually cut into small cross-sections (0.4 × 0.4 cm) and mounted on SEM pins using double-sided carbon tape. They were then coated with a 6 nm thick gold-palladium layer using a Leica EM ACE600 vacuum sputtering device (Leica Microsystems, Vienna, Austria). Both backscattered electron (BSE) and secondary electron (SE) detectors were used. Imaging was performed at 1.0 kV magnification and 5 kV accelerating voltage to accurately characterise the dimensions and characteristics.

2.20. Statistical analysis

Data were entered and stored in Microsoft Excel. Statistical analysis was performed using IBM SPSS software package version 29.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics included the mean and standard deviation of the investigated groups. Fatty acid composition was expressed as the percentage of the total identified fatty acid content. Data normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test before analysis of variance (ANOVA).

Data analysis was performed using analysis of variance (ANOVA). A three-way analysis of variance was applied to evaluate the effects of animal species, organs, and raw material category (edible offal and Category 3 animal by-products). The antioxidant activity of liver hydrolysates was assessed by analysing the effects of offal group, enzyme type, hydrolysis time, and their interactions. Statistical significance was determined using a three-way ANOVA model, followed by Tukey's HSD *post hoc* test ($p < 0.001$).

Two-way ANOVA was used to analyse the degree of hydrolysis (DH), evaluating the effects of hydrolysis time and enzyme type. Tukey's HSD *post hoc* test was applied to determine statistically significant differences ($p < 0.001$).

Pearson's correlation coefficient was used to assess the relationships between antioxidant activity parameters and the relative abundance of identified peptides, as determined from chromatographic peak areas ($p < 0.01$).

Gummy candy data were analysed using one-way ANOVA, and Tukey's HSD *post hoc* test was applied to determine statistically significant differences between sample means ($p < 0.05$).

3. RESULTS

3.1. Bovine, porcine and ovine post-mortem organ inspection

The distribution of pathological lesions in the organ systems of cattle identified during veterinary post-mortem examination is presented in Table 3.1.1. In cows, the most frequently observed lesions were those of the digestive system (10.09%), followed by cardiovascular (7.65%), respiratory (5.50%) and urinary system lesions (5.20%). In bulls, digestive system lesions were also the most common (12.35%), followed by respiratory (6.79%), urinary (6.17%) and cardiovascular lesions (6.17%). In heifers, digestive system lesions accounted for the highest proportion (4.50%), whereas respiratory and cardiovascular lesions were less frequent (2.70%. each), and no urinary system lesions were recorded.

No statistically significant differences were found between cattle categories for any of the examined organ systems, including urinary system lesions ($\chi^2 = 3.72$; $p = 0.15$), digestive system lesions ($\chi^2 = 2.56$; $p = 0.28$), respiratory system lesions ($\chi^2 = 1.34$; $p = 0.51$) and cardiovascular system lesions ($\chi^2 = 0.96$; $p = 0.62$).

Table 3.1.1. *Pathological lesions detected in bovine organ systems during veterinary post-mortem examination*

Organ system	Cows (n = 327), n (%)	Bulls (n = 162), n (%)	Heifers (n = 111), n (%)	χ^2	p
Respiratory system lesions	18 (5.50)	11 (6.79)	3 (2.70)	1.34	0.51
Digestive system lesions	33 (10.09)	20 (12.35)	5 (4.50)	2.56	0.28
Urinary system lesions	17 (5.20)	10 (6.17)	N.D.	3.72	0.15
Cardiovascular system lesions	25 (7.65)	10 (6.17)	3 (2.70)	0.96	0.62

N.D. – not detected. Data are presented as the number of lesions and percentage (%), n (%). Percentages were calculated based on the total number of animals in each group.

The pathological lesions detected in cattle during veterinary post-mortem examination are summarised in Table 3.1.2. Liver-related lesions were among the most common findings, with parasitic fascioliasis being the most prevalent (3.33%), followed by hepatic fatty degeneration (2.17%) and chronic cholangitis (1.67%). Among respiratory system lesions, pulmonary emphysema (2.33%), and pulmonary aspiration (2.00%).

Table 3.1.2. *Pathological lesions detected in bovines during veterinary post-mortem examination*

Lesion	Cows (n = 327), n (%)	Bulls (n = 162), n (%)	Heifers (n = 111), n (%)	Total (n)	Total (%)
Hepatic fatty degeneration	8 (1.33)	5 (0.83)	N.D.	13	2.17
Hepatic abscesses	3 (0.50)	2 (0.33)	1 (0.17)	6	1.00
Hepatic fasciolosis	12 (2.00)	5 (0.83)	3 (0.50)	20	3.33
Chronic cholangitis	5 (0.83)	4 (0.67)	1 (0.17)	10	1.67
Hepatic hyperaemia	5 (0.83)	3 (0.50)	N.D.	8	1.33
Focal hepatic necrosis	N.D.	1 (0.17)	N.D.	1	0.17
Fibrinous pericarditis	6 (1.00)	8 (1.33)	3 (0.50)	17	2.83
Endocarditis	9 (1.50)	2 (0.33)	N.D.	11	1.83
Myocardial hemorrhages	10 (1.67)	9 (1.50)	1 (0.17)	20	3.33
Pulmonary emphysema	9 (1.50)	5 (0.83)	3 (0.50)	14	2.33
Pulmonary aspiration	6 (1.00)	6 (1.00)	N.D.	12	2.00
Pneumonia	3 (0.50)	N.D.	N.D.	3	0.50
Chronic interstitial nephritis	9 (1.50)	9 (1.50)	N.D.	18	3.00
Renal cysts	8 (1.33)	1 (0.17)	N.D.	9	1.50
Total pathological lesions				162	27.00

N.D. – not detected. The percentage of all pathological lesions was calculated relative to the total number of animals examined.

The distribution of pathological lesions in the organ systems of porcine identified during veterinary post-mortem examination is presented in Table 3.1.3. After a veterinary post-mortem examination of 600 porcine carcasses, pathological lesions were detected in 81 carcasses (13.50%), while no pathological lesions were detected in the remaining 519 carcasses (86.50%). Cardiovascular system lesions were most frequently recorded, detected in 4.83% of all examined carcasses. Kidney lesions were detected in 20 cases (3.33%), while respiratory and cardiovascular lesions were detected equally often, at 17 cases each (2.83%).

Table 3.1.3. *Pathological changes detected in porcine organ systems during veterinary post-mortem examination*

Organ system	Porcine (n = 600), n (%)*
Respiratory system lesions	17 (2.83)
Digestive system lesions	27 (4.50)
Urinary system lesions	20 (3.33)
Cardiovascular system lesions	29 (4.83)

*The percentage of lesions was calculated based on the total number of porcine examined.

The pathological lesions detected in porcine during the veterinary post-mortem examination are summarized in Table 3.1.4. A more detailed analysis of the findings showed that the most frequently diagnosed pathology was renal cysts, detected in 20 animals (3.33%). Liver pathological changes were mainly characterized by fatty liver degeneration (13 cases; 2.17%) and liver necrosis (11 cases; 1.83%), while liver abscesses were found less frequently (3 cases; 0.50%). In the respiratory system, the most frequently diagnosed condition was chronic bronchopneumonia (9 cases; 1.50%), followed by aspiration pneumonia (8 cases; 1.33%) and pulmonary emphysema (9 cases; 1.50%). The most common cardiovascular lesions were myocardial haemorrhage (12 cases; 2.00%), followed by endocarditis (10 cases; 1.67%) and fibrinous pericarditis (7 cases; 1.17%).

Table 3.1.4. *Pathological lesions detected in porcine during veterinary post-mortem examination*

Lesion	Porcine (n = 600), n (%)*
Hepatic fatty degeneration	13 (2.17)
Hepatic necrosis	11 (1.83)
Hepatic abscesses	3 (0.5)
Fibrinous pericarditis	7 (1.17)
Endocarditis	10 (1.67)
Myocardial hemorrhages	12 (2.00)
Chronic bronchopneumonia	9 (1.50)
Aspiration pneumonia	8 (1.33)
Pulmonary emphysema	9 (1.50)
Renal cysts	20 (3.33)
Total pathological lesions	93 (15.50)

*The percentage of lesions was calculated based on the total number of porcine examined.

The distribution of pathological lesions in the organ systems of the ovine identified during veterinary post-mortem examination is presented in Table 3.1.5. After a veterinary post-mortem inspection of 600 ovine carcasses, pathological lesions were detected in 61 carcasses (10.17%). In comparison, no pathological lesions were observed in the remaining 539 carcasses (89.83%). Liver lesions were most frequently recorded, detected in 33 carcasses, accounting for 5.50% of all examined animals. Urinary system lesions were identified in 10 cases (1.67%), while respiratory and cardiovascular system lesions were detected equally often, with 9 cases recorded in each group (1.50%).

Table 3.1.5. *Pathological lesions detected in ovine organ systems during veterinary post-mortem examination*

Organ system	**Ovine (n = 600), n (%)***
Respiratory system lesions	9 (1.50)
Digestive system lesions	33 (5.50)
Urinary system lesions	10 (1.67)
Cardiovascular system lesions	9 (1.50)

*The percentage of lesions was calculated based on the total number of ovine examined.

The pathological lesions detected in ovine during the veterinary post-mortem examination are summarized in Table 3.1.6. A detailed analysis of the pathologic lesions revealed that the most frequently diagnosed liver pathology was liver fibrosis, identified in 14 animals (2.33%), followed by fatty liver degeneration (9 cases; 1.50%) and liver abscesses (8 cases; 1.33%). Liver fasciolosis was less common and was detected in only 2 animals (0.33%).

In the respiratory system, pulmonary emphysema was the only pathological finding, diagnosed in 9 carcasses (1.50%). Cardiovascular system lesions were represented by myocardial haemorrhages, mechanical damage detected in 9 animals (1.50%). Among urinary system pathologies, renal cysts were more frequently observed (8 cases; 1.33%), whereas nephritis was diagnosed in only 2 animals (0.33%).

Table 3.1.6. *Pathological lesions detected in ovine during veterinary post-mortem examination*

Lesion	Ovine (n = 600), n (%)*
Hepatic fibrosis	14 (2.33)
Hepatic fasciolosis	2 (0.33)
Hepatic fatty degeneration	9 (1.50)
Hepatic abscesses	8 (1.33)
Pulmonary emphysema	9 (1.50)
Myocardial hemorrhages	9 (1.50)
Nephritis	2 (0.33)
Renal cysts	8 (1.33)
Total ante mortem pathological lesions	61 (10.17)

*The percentage of lesions was calculated based on the total number of ovine examined.

3.2. Nutritional composition of lyophilised edible offal and Category 3 animal by-products

3.2.1. Protein content

The protein content of freeze-dried edible offal and Category 3 animal by-products is presented in Fig. 3.2.1. It was found that the organ type, animal species and offal group had a statistically significant influence on the protein content ($p < 0.05$). The highest protein content was found in liver samples (16.3–19.3 ± 0.01%), heart samples also recorded high protein content (15.4 ± 0.01–17.2 ± 0.02%), and the lowest in lung samples (12.3 ± 0.02–15.8 ± 0.02%). Within the same offal and offal group, significant differences in protein content were found between animal species ($p < 0.05$). The largest differences were found in the liver and kidneys, where the protein content of edible sheep offal was higher than that of the bovine and porcine offal. Significant differences were also found between edible offal and Category 3 by-products when evaluating samples of the same offal from different animal species ($p < 0.05$). In all organs tested, the protein content in edible offal samples was statistically significantly higher than in Category 3 (C3) animal by-products.

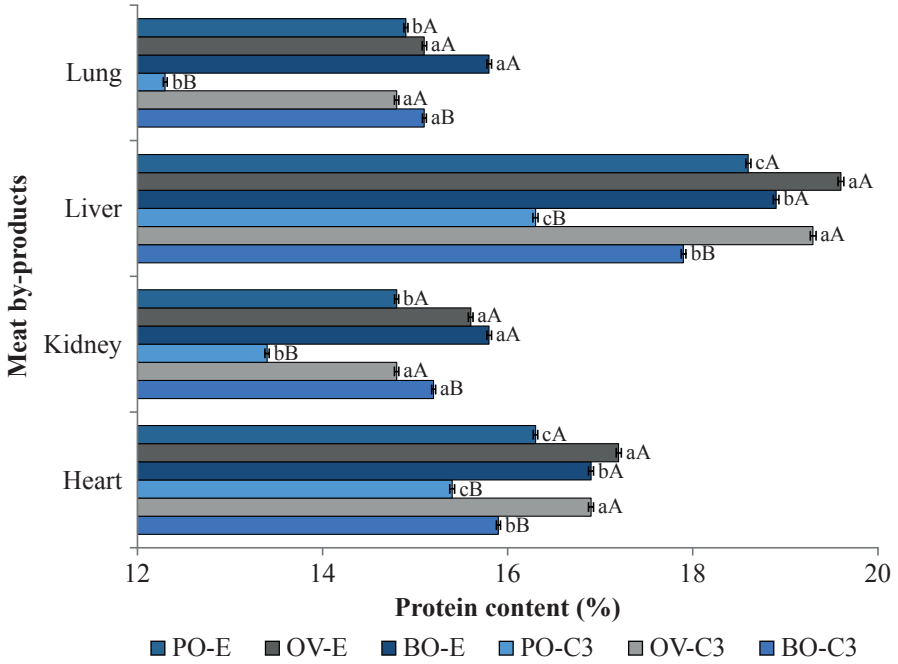


Fig. 3.2.1.1. *The protein content of lyophilised edible offal and Category 3 animal by-products*

Different lowercase letters (a–c) within the same offal and category indicate statistically significant differences among species ($p < 0.05$). Different uppercase letters (A–B) within the same offal and species indicate statistically significant differences between Category 3 animal by-products and edible offal ($p < 0.05$). The results text analyses statistically significant differences between edible offal (E) and Category 3 animal by-products (C3). PO – porcine; OV – ovine; BO – bovine; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009.

3.2.2. Fat content

The fat content of lyophilized edible offal and Category 3 animal by-products is presented in Fig. 3.2.2.1. It was determined that both organ type and animal species had a statistically significant effect on fat content ($p < 0.05$). The highest fat content was found in porcine heart ($8.4 \pm 0.02\%$) and ovine liver ($5.2 \pm 0.01\%$), reflecting pronounced differences related to organ type and species.

When comparing edible offal with Category 3 animal by-products, it was observed that fat content differed significantly in lung and kidney samples ($p < 0.05$), whereas no statistically significant differences between these categories were found in liver and heart samples.

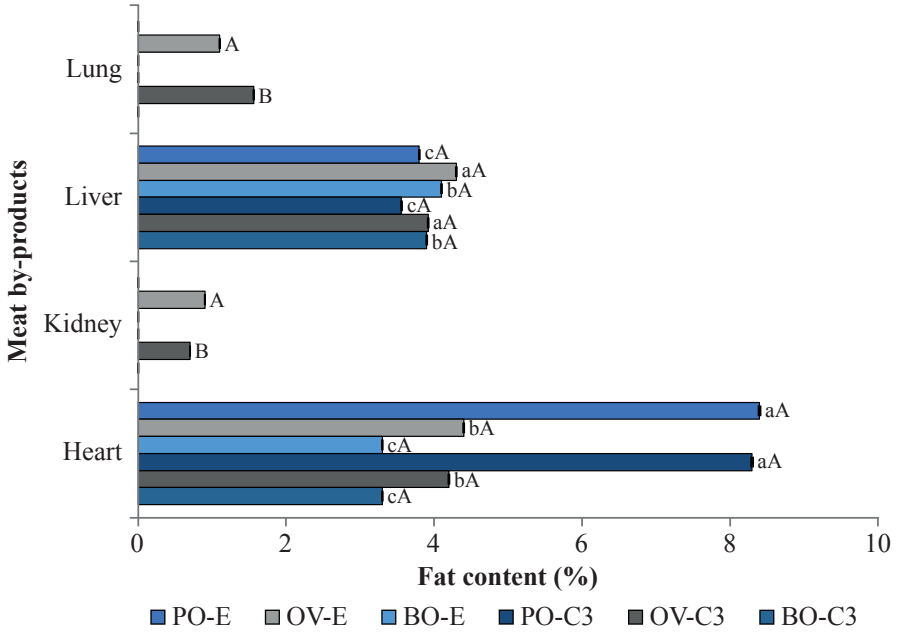


Fig. 3.2.2.1. *The fat content of lyophilised edible offal and Category 3 animal by-products*

Different lowercase letters (a–c) within the same offal and offal group indicate statistically significant differences among species ($p < 0.05$). Different uppercase letters (A–B) within the same offal and species indicate statistically significant differences between Category 3 animal by-products and edible offal ($p < 0.05$). The results text analyses statistically significant differences between edible offal (E) and Category 3 (C3) animal by-products. PO – porcine; OV – ovine; BO – bovine; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009.

3.2.3. Fatty acid composition and lipid quality indices

The fatty acid (FA) groups and lipid quality indices (LQIs) of edible offal (E) and Category 3 (C3) animal by-products are presented in Table 3.2.3.1. In animal lung samples, saturated fatty acids (SFA) constituted the largest proportion of total fatty acids and ranged from 53.85% to 56.20% in edible offal samples and from 56.55% to 58.87% in C3 samples ($p < 0.05$). Polyunsaturated fatty acids (PUFA) content was highest in porcine lung (23.79 ± 0.05%) and lowest in ovine lung (11.90 ± 0.05%) ($p < 0.05$). C3 lung samples were characterized by a higher omega-6/omega-3 ratio (up to 14.57) and higher atherogenicity (AI) and thrombogenicity (TI) indices (up to 2.47) compared to edible samples ($p < 0.05$).

In liver samples, SFA also constituted the largest proportion of total fatty acids and ranged from 50.70% to 56.76% in edible offal and from 50.72% to 53.14% in C3 samples ($p < 0.05$). The content of PUFA differed significantly

between species ($p < 0.05$) and was highest in porcine liver (26.69 ± 0.05%), while PUFA content was lower in ovine and bovine liver (12.11% and 10.20%, respectively). The content of omega-6 fatty acids was similar and reached (23.46 ± 0.04%) in porcine liver, but remained significantly lower in ovine samples (5.13%) ($p < 0.05$).

C3 liver samples generally had higher PUFA content (24.16–28.03%) and PUFA/SFA ratio (0.46–0.55) compared to edible samples ($p < 0.05$). Higher (h/H) ratios (2.06–2.84) were observed in C3 liver than in edible offal (0.47–1.32) ($p < 0.05$), indicating differences in lipid quality between offal groups.

In kidney samples, SFA constituted the largest proportion of total fatty acids and ranged from 46.53% to 60.89% in edible offal and from 57.10% to 61.32% in C3 samples ($p < 0.05$). The PUFA content differed significantly between species ($p < 0.05$) and was highest in bovine kidneys (25.71 ± 0.03%), while lower values were observed in porcine and ovine kidneys. Bovine kidney samples were also determined to have significantly higher levels of omega-3 fatty acids (17.93 ± 0.05%), whereas porcine and ovine samples contained considerably lower amounts ($p < 0.05$). Bovine kidneys showed a more favourable PUFA/SFA ratio (0.55) and a lower thrombogenicity index (0.59) compared to porcine and ovine kidneys ($p < 0.05$). In C3 kidney samples, the PUFA content ranged from 15.02% to 20.72% , while the PUFA/SFA ratio varied between 0.26 and 0.34. The h/H ratio was also higher in C3 samples (1.48–1.83) than in edible offal (0.46–1.18) ($p < 0.05$), indicating differences in lipid quality between product categories.

In heart samples, the largest proportion of total fatty acids was composed of saturated fatty acids (SFA), which ranged from 41.11% to 62.18% in edible offal and from 57.04% to 58.66% in C3 samples ($p < 0.05$). The content of polyunsaturated fatty acids (PUFA) differed significantly between species ($p < 0.05$) and was highest in ovine C3 samples (21.15 ± 0.06%) and lowest in ovine edible offal (7.50 ± 0.02%). Porcine heart samples were characterized by a higher content of omega-6 fatty acids and a higher n-6/n-3 ratio (up to 15.77), while this ratio was significantly lower in ovine and bovine samples ($p < 0.05$). Lipid quality indicators differed significantly, with higher atherogenicity (AI) and thrombogenicity (TI) indices being found in ovine and bovine samples, while porcine samples had a more favorable lipid profile. The PUFA/SFA ratio was highest in porcine edible samples (0.42) and lowest in ovine samples (0.12). Higher h/H ratios (1.43–1.81) were found in C3 samples compared to edible offal (0.27–0.73) ($p < 0.05$), indicating differences between offal groups.

Table 3.2.3.1. *Fatty acid (FA) composition and lipid quality indices of lyophilized edible offal (E) and Category 3 (C3) animal by-products*

Sample		SFA	MUFA	PUFA	UFA	Omega-3	Omega-6	Omega-6/3	PUFA/SFA	AI	TI	h/H
Lungs	Lu-PO-E	54.84^{bB}	21.46^{cB}	23.79^{aB}	45.25^{bB}	5.63^{bB}	18.16^{aB}	3.22^{bB}	0.43^{aB}	0.73^{bB}	1.47^{bB}	0.92^{aB}
	Lu-OV-E	56.20^{aB}	31.98^{aB}	11.90^{cB}	43.88^{cB}	6.54^{aB}	5.36^{cB}	0.82^{cB}	0.21^{cB}	0.64^{cB}	1.37^{cB}	0.50^{cB}
	Lu-BO-E	53.85^{cB}	28.05^{bB}	18.10^{bB}	46.15^{aB}	3.64^{cB}	14.46^{bB}	3.97^{aB}	0.34^{bB}	0.67^{aB}	1.62^{aB}	0.74^{bB}
	Lu-PO-C3	58.87^{aA}	25.11^{bA}	16.02^{cA}	41.13^{cA}	1.19^{cA}	14.83^{bA}	12.46^{bA}	0.27^{bA}	0.91^{aA}	2.47^{aA}	1.42^{aA}
	Lu-OV-C3	57.35^{bA}	25.33^{bA}	17.32^{bA}	42.65^{bA}	3.10^{aA}	14.12^{bA}	4.55^{cA}	0.30^{aA}	0.80^{bA}	1.96^{cA}	1.60^{aA}
	Lu-BO-C3	56.55^{cA}	25.11^{bA}	18.53^{aA}	43.64^{aA}	1.19^{cA}	17.34^{aA}	14.57^{aA}	0.33^{aA}	0.81^{bA}	2.26^{bA}	1.58^{aA}
	Mean ± SD	0.04	0.04	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.05
Liver	Li-PO-E	50.70^{cB}	22.62^{cB}	26.69^{aB}	49.30^{aB}	3.22^{bB}	23.46^{aB}	7.28^{aB}	0.53^{aB}	0.43^{bB}	1.51^{bB}	1.32^{bB}
	Li-OV-E	56.76^{aB}	31.11^{bB}	12.11^{bB}	43.23^{cB}	6.99^{aB}	5.13^{cB}	0.73^{cB}	0.21^{bB}	0.52^{aB}	1.24^{cB}	0.60^{cB}
	Li-BO-E	55.99^{bB}	33.82^{aB}	10.20^{cB}	44.01^{bB}	2.22^{cB}	7.97^{bB}	3.59^{bB}	0.18^{cB}	0.55^{aB}	1.96^{aB}	0.47^{cB}
	Li-PO-C3	53.14^{aA}	21.43^{bA}	25.43^{bA}	46.86^{cA}	4.22^{bA}	21.21^{aA}	5.03^{bA}	0.48^{bA}	0.47^{bA}	1.52^{bA}	2.25^{bA}
	Li-OV-C3	50.72^{cA}	21.24^{bA}	28.03^{aA}	49.27^{aA}	7.68^{aA}	20.35^{cA}	2.65^{cA}	0.55^{aA}	0.44^{cA}	1.09^{cA}	2.84^{aA}
	Li-BO-C3	52.50^{bA}	23.43^{aA}	24.16^{bA}	47.59^{cA}	3.23^{bA}	20.93^{bA}	6.48^{aA}	0.46^{cA}	0.50^{aA}	1.61^{aA}	2.06^{cA}
	Mean ± SD	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Kidney	K-PO-E	60.89^{aB}	28.76^{bB}	10.53^{bB}	39.29^{cB}	3.46^{cB}	7.08^{bB}	2.05^{bB}	0.17^{cB}	0.70^{bB}	1.95^{bB}	0.46^{cB}
	K-OV-E	60.89^{aB}	29.40^{aB}	9.50^{cB}	38.90^{bB}	4.43^{bB}	5.08^{cB}	1.15^{cB}	0.16^{cB}	0.71^{aB}	1.78^{bB}	0.41^{cB}
	K-BO-E	46.53^{bB}	27.75^{cB}	25.71^{aB}	53.47^{aB}	17.93^{aB}	7.78^{aB}	0.43^{aB}	0.55^{aB}	0.44^{cB}	0.59^{cB}	1.18^{bB}
	K-PO-C3	57.10^{cA}	27.88^{aA}	15.02^{bA}	42.90^{bA}	1.06^{bA}	13.96^{bA}	13.13^{aA}	0.26^{bA}	0.67^{bA}	2.31^{aA}	1.66^{aA}
	K-OV-C3	61.32^{aA}	18.03^{cA}	20.72^{cA}	38.75^{cA}	4.47^{bA}	16.25^{aA}	3.64^{cA}	0.34^{bA}	0.65^{cA}	1.82^{bA}	1.83^{aA}
	K-BO-C3	59.57^{bA}	25.22^{bA}	15.24^{aA}	40.46^{aA}	1.41^{aA}	13.83^{cA}	9.81^{bA}	0.26^{bA}	0.74^{aA}	2.44^{aA}	1.48^{aA}
	Mean ± SD	0.03	0.02	0.02	0.04	0.03	0.03	0.03	0.03	0.04	0.04	0.04

Table 3.2.3.1. *Continued*

Sample		SFA	MUFA	PUFA	UFA	Omega-3	Omega-6	Omega-6/3	PUFA/SFA	AI	TI	h/H
Heart	H-PO-E	41.11^{cB}	42.23^{aB}	17.10^{bB}	59.32^{aB}	1.02^{bB}	16.08^{aB}	15.77^{aB}	0.42^{aB}	0.45^{bB}	1.26^{bB}	0.73^{aB}
	H-OV-E	62.18^{aB}	31.19^{bB}	7.50^{cB}	38.69^{cB}	2.37^{cB}	5.13^{cB}	2.17^{cB}	0.12^{cB}	0.97^{aB}	2.35^{cB}	0.27^{cB}
	H-BO-E	59.82^{bB}	30.62^{cB}	9.56^{aB}	40.18^{bB}	3.05^{bB}	6.51^{bB}	2.13^{bB}	0.16^{bB}	0.86^{bB}	2.07^{aB}	0.34^{bB}
	H-PO-C3	57.04^{bA}	26.38^{bA}	16.67^{bA}	43.05^{bA}	1.07^{bA}	15.60^{bA}	14.58^{bA}	0.29^{bA}	0.84^{bA}	2.33^{bA}	1.61^{bA}
	H-OV-C3	58.66^{aA}	20.24^{cA}	21.15^{cA}	41.39^{cA}	4.71^{aA}	16.44^{aA}	3.49^{cA}	0.36^{aA}	0.76^{cA}	1.88^{cA}	1.81^{aA}
	H-BO-C3	57.89^{cA}	27.58^{aA}	14.53^{aA}	42.11^{aA}	0.92^{cA}	13.61^{cA}	14.79^{aA}	0.25^{cA}	0.85^{aA}	2.45^{aA}	1.43^{cA}
	Mean ± SD	0.04	0.04	0.02	0.02	0.03	0.03	0.03	0.03	0.04	0.04	0.04

Different lowercase letters ($^{a–c}$) within the same offal and offal group indicate statistically significant differences among species ($p < 0.05$). Different uppercase letters ($^{A–B}$) within the same offal and species indicate statistically significant differences between Category 3 animal by-products (C3) and edible offal (E) ($p < 0.05$). Organ type effects are described in the text. Lu – lungs; Li – liver; K – kidney; H – heart; PO – porcine; OV – ovine; BO – bovine; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009.

3.2.4. Amino acid content

The amino acid profiles of lyophilised edible offal (E) and Category 3 (C3) animal by-products were assessed using the heat map visualization in Figure 3.2.4.1. The heat map revealed differences in amino acid composition between edible offal and Category 3 animal by-products. For all animal species and organ types, the most abundant amino acids were glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), glycine (Gly) and leucine (Leu), while the aromatic amino acids phenylalanine and tyrosine were present in lower concentrations and more evenly distributed.

Hierarchical cluster analysis of amino acid concentrations demonstrated that sample differentiation was primarily associated with organ type rather than animal species or offal group (edible offal and Category 3 animal by-products). Liver samples formed a distinct cluster characterized by higher total amino acid concentrations, especially glutamic acid, aspartic acid, leucine and alanine. The second main cluster consisted of heart, kidney and lung samples, which were further divided into organ-specific subclusters. Lung samples clustered independently within this group, reflecting higher concentrations of glycine and alanine, while heart and kidney samples were more closely related and characterized by intermediate amino acid concentrations.

Three-way analysis of variance showed that organ type was the main factor determining the differences in the concentrations of all amino acids tested ($p < 0.001$). Glutamic acid was the dominant amino acid in all samples tested, regardless of animal origin, organ type, or offal group. Its concentration depended on organ type, which was particularly strong for the distribution of glutamic acid ($F = 329.45$, $p < 0.001$).

The concentrations of essential amino acids also differed significantly by organ type, and a test was performed to determine the distribution of all essential amino acids tested ($p < 0001$). The highest total concentrations of these amino acids were found in samples that significantly exceeded the values in the heart and lungs. Among the essential amino acids, leucine dominated in all tested organs, with the highest concentrations in the liver and the lowest in the heart and lungs ($p < 0.001$). A similar organ-related tendency was observed for isoleucine, while methionine showed the most pronounced organ-specific differences: significantly higher concentrations were found in liver samples, and the lowest in heart tissues ($p < 0.001$). Threonine concentrations were also significantly influenced by offal type ($p < 0.001$), with the highest concentrations observed in liver and kidney samples and the lowest in lung samples. Meanwhile, differences in valine concentrations among organs were less pronounced, while animal species had

a significant effect on valine concentrations, with higher values observed in bovine samples than in porcine and ovine samples ($p < 0.05$).

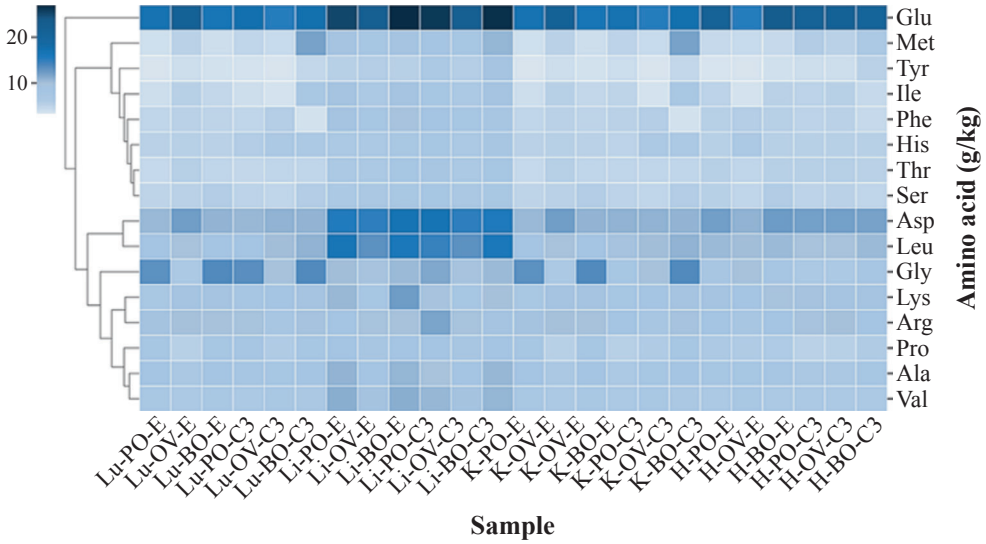


Fig. 3.2.4.1. *Amino acid profiles of lyophilised edible offal (E) and Category 3 (C3)animal by-products across different sample groups (g/kg of dry matter)*

Lu – lung; Li – liver; K – kidney; H – heart; PO – porcine; OV – ovine; BO – bovine; E – edible offal; C3 – Category 3 animal by-products.

3.3. Microbiological and chemical safety parameters of lyophilised edible offal and Category 3 animal by-products

The analysis of microbiological indicators is presented in Table 3.3.1, which summarises the microbiological quality of lyophilized edible offal and Category 3 animal by-products. Animal species within the same offal type and offal group showed statistically significant differences in total aerobic microbial count (TAMC) ($p < 0.05$). Although no significant differences were found between porcine and bovine lung samples, their TAMC values were significantly higher than those of ovine lung samples in both offal groups. Statistically significant differences were also found between animal species in liver samples in both offal groups ($p < 0.05$). In the edible offal group, porcine and ovine liver TAMC values were higher than those of bovine liver samples, whereas in the Category 3 offal group, ovine liver samples had the highest TAMC values. In kidney samples, ovine and bovine TAMC values were significantly higher than those of porcine samples in both offal groups ($p < 0.05$). In both the edible and Category 3 offal groups, the TAMC values

of porcine hearts were significantly higher than those of ovine and bovine hearts ($p < 0.05$), while no significant differences were found between bovine and ovine hearts in the Category 3 offal group. Regardless of animal species or offal group, the levels of *Escherichia coli* in all offal samples tested were below the detection limit (< 10 CFU/g). *Enterobacteriaceae* were also below the detection limit in most samples but were occasionally detected in liver samples, with levels ranging from 2.16 ± 0.12 to 2.26 ± 0.14 $\log_{10}$ CFU/g. *Listeria monocytogenes* and *Salmonella* spp. were not detected in any of the offal samples tested.

Table 3.3.1. *Microbiological quality of lyophilised edible offal (E) and Category 3 (C3) animal by-products*

Sample code	**Total aerobic microbial count (TAMC) ($\log_{10}$ CFU/g)**	***E. coli*** **($\log_{10}$ CFU/g)**	***Enterobacteriaceae*** **($\log_{10}$ CFU/g)**	***Salmonella*** **spp.**	***Listeria monocytogenes***
Lu-PO-E	4.48 ± 0.15^{a}	< 10	< 10	N.D.	N.D.
Lu-OV-E	2.30 ± 0.18^{b}	< 10	< 10	N.D.	N.D.
Lu-BO-E	4.60 ± 0.15^{a}	< 10	< 10	N.D.	N.D.
Lu-PO-C3	4.62 ± 0.04^{a}	< 10	< 10	N.D.	N.D.
Lu-OV-C3	2.51 ± 0.05^{b}	< 10	< 10	N.D.	N.D.
Lu-BO-C3	4.65 ± 0.16^{a}	< 10	< 10	N.D.	N.D.
Li-PO-E	3.85 ± 0.12^{a}	< 10	2,16 ± 0,12	N.D.	N.D.
Li-OV-E	3.18 ± 0.04^{b}	< 10	< 10	N.D.	N.D.
Li-BO-E	2.30 ± 0.08^{c}	< 10	< 10	N.D.	N.D.
Li-PO-C3	3.91 ± 0.15^{b}	< 10	2,26 ± 0,14	N.D.	N.D.
Li-OV-C3	4.65 ± 0.07^{a}	< 10	< 10	N.D.	N.D.
Li-BO-C3	2.75 ± 0.16^{c}	< 10	2,18 ± 0,06	N.D.	N.D.
K-PO-E	3.41 ± 0.06^{b}	< 10	< 10	N.D.	N.D.
K-OV-E	3.65 ± 0.19^{a}	< 10	< 10	N.D.	N.D.
K-BO-E	3.57 ± 0.06^{a}	< 10	< 10	N.D.	N.D.
K-PO-C3	3.49 ± 0.09^{b}	< 10	< 10	N.D.	N.D.
K-OV-C3	3.65 ± 0.07^{a}	< 10	< 10	N.D.	N.D.
K-BO-C3	3.61 ± 0.15^{a}	< 10	< 10	N.D.	N.D.

Table 3.3.1. *Continued*

Sample	Total aerobic microbial count (TAMC) (log_{10} CFU/g)	*E. coli* (log_{10} CFU/g)	*Enterobacteriaceae* (log_{10} CFU/g)	*Salmonella* spp.	*Listeria monocytogenes*
H-PO-E	4.46 ± 0.08^{a}	< 10	< 10	N.D.	N.D.
H-OV-E	3.30 ± 0.12^{b}	< 10	< 10	N.D.	N.D.
H-BO-E	3.11 ± 0.04^{c}	< 10	< 10	N.D.	N.D.
H-PO-C3	4.45 ± 0.16^{a}	< 10	< 10	N.D.	N.D.
H-OV-C3	3.30 ± 0.04^{b}	< 10	< 10	N.D.	N.D.
H-BO-C3	3.36 ± 0.06^{b}	< 10	< 10	N.D.	N.D.

Different lowercase letters ($^{a–c}$) indicate statistically significant differences among species within the same offal and group ($p < 0.05$). N.D. – not detected. Lu – lung; Li – liver; K – kidney; H – heart; PO – porcine; OV – ovine; BO – bovine; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009.

The results of organochlorine pesticide (OCP) analysis in edible offal and Category 3 animal by-products are presented in Table 3.3.2. The concentrations of all analyzed organochlorine pesticides were below the limit of detection (LOD) in all samples. No residues of aldrin, dieldrin, heptachlor epoxide, chlordanes, or DDT and its metabolites were detected.

Table 3.3.2. *Assessment of organochlorine pesticide (OCP) residues in edible offal (E) and Category 3 (C3) animal by-products*

Sample code	Aldrin	Dieldrin	Heptachlor epoxide	Σ Chlordanes	Σ DDTs
Lu-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Li-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Li-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
Li-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Li-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Li-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Li-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD

Table 3.3.2. *Continued*

Sample	Aldrin	Dieldrin	Heptachlor epoxide	Σ Chlordanes	Σ DDTs
K-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
K-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
K-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
K-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
K-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
K-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
H-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
H-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
H-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
H-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
H-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
H-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD

Lu – lung; Li – liver; K – kidney; H – heart; PO – porcine; OV – ovine; BO – bovine; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009; LOD – limit of detection.

The concentrations of mercury (Hg), cadmium (Cd) and lead (Pb) in edible offal (E) and Category 3 (C3) by-products of different animal organs are presented in Table 3.3.3. The concentrations of Hg and Pb in all samples tested were below the limit of detection (LOD). Cd was detected in liver and kidney samples, while in lung and heart samples its concentrations remained below the limit of detection (LOD). The highest Cd concentrations were found in kidney (0.41–0.58 mg/kg), which were significantly higher than in liver samples (0.06–0.09 mg/kg) ($p < 0.05$). No statistically significant differences were found between edible offal and Category 3 (C3) by-products ($p > 0.05$).

Table 3.3.3. *Concentrations of heavy metals (Hg, Cd and Pb) in edible offal (E) and Category 3 (C3) animal by-products*

Sample code	Mercury (mg/kg)	Cadmium (mg/kg)	Lead (mg/kg)
Lu-PO-E	< LOD	< LOD	< LOD
Lu-OV-E	< LOD	< LOD	< LOD
Lu-BO-E	< LOD	< LOD	< LOD
Lu-PO-C3	< LOD	< LOD	< LOD
Lu-OV-C3	< LOD	< LOD	< LOD
Lu-BO-C3	< LOD	< LOD	< LOD
Li-PO-E	< LOD	0.08 ± 0.01^{a}	< LOD
Li-OV-E	< LOD	0.06 ± 0.01^{a}	< LOD
Li-BO-E	< LOD	0.08 ± 0.01^{a}	< LOD
Li-PO-C3	< LOD	0.07 ± 0.01^{a}	< LOD
Li-OV-C3	< LOD	0.09 ± 0.01^{a}	< LOD
Li-BO-C3	< LOD	0.08 ± 0.01^{a}	< LOD
K-PO-E	< LOD	0.42 ± 0.07^{b}	< LOD
K-OV-E	< LOD	0.56 ± 0.04^{b}	< LOD
K-BO-E	< LOD	0.48 ± 0.07^{b}	< LOD
K-PO-C3	< LOD	0.41 ± 0.04^{b}	< LOD
K-OV-C3	< LOD	0.58 ± 0.08^{b}	< LOD
K-BO-C3	< LOD	0.45 ± 0.08^{b}	< LOD
H-PO-E	< LOD	< LOD	< LOD
H-OV-E	< LOD	< LOD	< LOD
H-BO-E	< LOD	< LOD	< LOD
H-PO-C3	< LOD	< LOD	< LOD
H-OV-C3	< LOD	< LOD	< LOD
H-BO-C3	< LOD	< LOD	< LOD

Different lowercase letters ($^{a–b}$) indicate statistically significant differences between organs ($p < 0.05$). Lu – lung; Li – liver; K – kidney; H – heart; PO – porcine; OV – ovine; BO – bovine; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009; LOD – limit of detection.

3.4. Effect of enzymatic treatment on the degree of hydrolysis of liver hydrolysates

Enzymatic treatment the degree of hydrolysis (DH) of liver samples (E and C3) is presented in Fig 3.4.1. The degree of hydrolysis (DH) of porcine liver proteins (Fig. 3.4.1 A) was significantly affected by enzyme treatment, hydrolysis time and their interaction ($p < 0.001$). DH values increased significantly with increasing hydrolysis time ($p < 0.001$). The highest degree of hydrolysis was observed in all samples after 24 h. After 3 h, DH values ranged from 8.28 ± 0.21% to 11.34 ± 0.85%. After 6 h of hydrolysis, DH increased to 13.28 ± 0.24%–20.23 ± 0.25%, and after 24 h, values reached 26.28 ± 0.44%–35.22 ± 0.33%.

The DH of ovine liver proteins (Fig. 3.4.1 B) was significantly affected by enzyme treatment, hydrolysis time and their interaction ($p < 0.001$). DH values increased significantly with increasing hydrolysis time ($p < 0.001$). After 3 h, DH ranged from 5.88 ± 0.33% to 12.21 ± 0.52%, and after 6 h, it increased to 11.52 ± 0.46%–19.23 ± 0.36%. The highest DH values were recorded after 24 h of hydrolysis (23.28 ± 0.62%–33.12 ± 0.42%).

A similar tendency was observed for bovine liver proteins (Fig. 3.4.1 C), where DH was significantly affected by enzyme treatment, hydrolysis time and their interaction ($p < 0.001$). After 3 h, DH values ranged from 7.18 ± 0.35% to 10.88 ± 0.40%. After 6 h, DH increased to 13.30 ± 0.42%–20.25 ± 0.42%, and after 24 h, the values ranged from 24.18 ± 0.60% to 34.12 ± 0.52%.

Significant differences between enzyme treatments were observed at all hydrolysis times in all samples ($p < 0.001$). For all substrates, papain treatment had the highest DH values, while flavourzyme treatment had significantly lower DH values. Pepsin-treated samples had intermediate DH values.

At this stage of the study, papain and pepsin were selected for further analysis due to their favourable degree of hydrolysis.

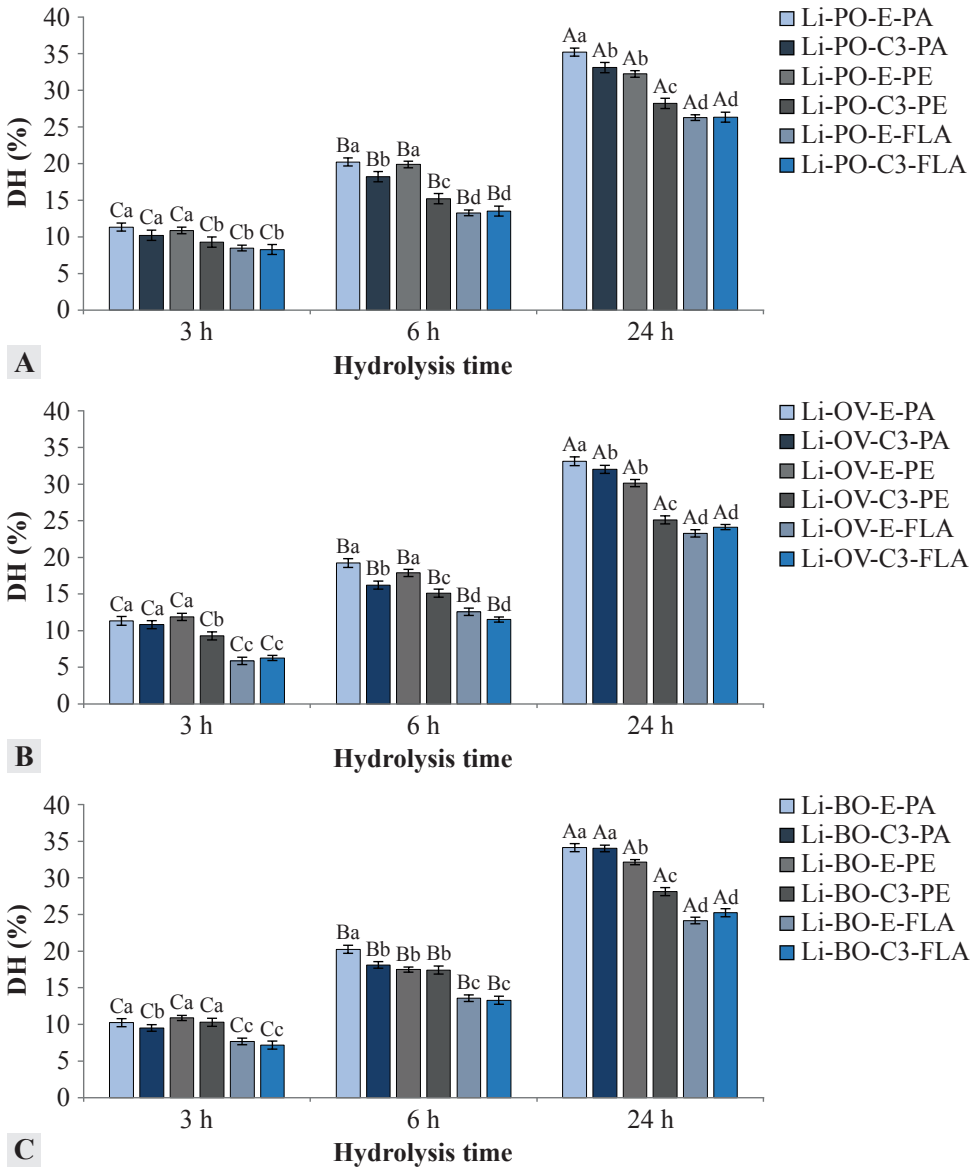


Fig. 3.4.1. *Determination of the degree of hydrolysis (DH) of liver hydrolysates using pepsin and papain at different hydrolysis times*

(A) Porcine liver; **(B)** Ovine liver; **(C)** Bovine liver. Different uppercase letters (A–C) within the same sample indicate statistically significant differences among hydrolysis times ($p < 0.001$). Different lowercase letters (a–d) within the same hydrolysis time indicate statistically significant differences between enzyme treatments ($p < 0.001$). Li – liver; BO – bovine; PA – papain; PE – pepsin; FLA – flavourzyme; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009.

3.5. Antioxidant activity ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays) of liver hydrolysates

The antioxidant properties ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging activity) of porcine, ovine, and bovine liver hydrolysates obtained using different enzymes after 3, 6, and 24 h of hydrolysis are presented in Fig. 3.5.1. The antioxidant activity of porcine liver hydrolysates was assessed using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays (Fig. 3.5.1 A). It significantly varied depending on the enzyme treatment, offal group (E and C3), hydrolysis time and their interaction ($p < 0.001$). The hydrolysis time had a pronounced effect on the radical scavenging activity, and the values increased significantly from 3 h to 24 h ($p < 0.001$). In the $ABTS^{\bullet+}$ assay, the highest scavenging activity was observed after 24 h of hydrolysis, reaching 97.20 ± 1.79% in edible samples and 94.20 ± 1.59% in Category 3 samples after papain treatment. Similar results were obtained in the $DPPH^{\bullet}$ assay, with maximum values being determined after 24 h, reaching 92.07 ± 2.23% (E) and 88.33 ± 2.01% (C3). Papain-treated liver hydrolysates exhibited significantly higher antioxidant activity at different hydrolysis times compared to pepsin-treated hydrolysates ($p < 0.001$). Liver hydrolysates from the edible offal group showed slightly higher radical scavenging activity than those from the Category 3 offal group, but hydrolysis time had the greatest influence on antioxidant activity.

The antioxidant activity of ovine liver hydrolysates was also evaluated using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays (Fig. 3.5.1 B). Antioxidant activity was significantly influenced by enzyme treatment, offal group (E and C3), hydrolysis time, and the interaction of these factors ($p < 0.001$). Hydrolysis time significantly increased radical scavenging activity from 3 h to 24 h ($p < 0.001$). In the $ABTS^{\bullet+}$ assay, after 3 h of hydrolysis, radical scavenging activity reached 19.21 ± 1.26% (E) and 17.52 ± 0.81% (C3) in papain-treated liver hydrolysates, and 10.27 ± 1.57% (E) and 11.44 ± 1.43% (C3) in pepsin-treated liver hydrolysates. After 6 h, the activity increased to 42.50 ± 1.35% (E) and 36.85 ± 1.87% (C3) in papain-treated liver hydrolysates, and to 39.41 ± 1.46% (E) and 36.28 ± 1.01% (C3) in pepsin-treated liver hydrolysates. The highest $ABTS^{\bullet+}$ activity was observed after 24 h, reaching 90.24 ± 2.01% (E) and 92.12 ± 1.59% (C3) in papain-treated liver hydrolysates, while pepsin-treated liver hydrolysates reached 89.10 ± 1.81% (E) and 70.10 ± 1.26% (C3).

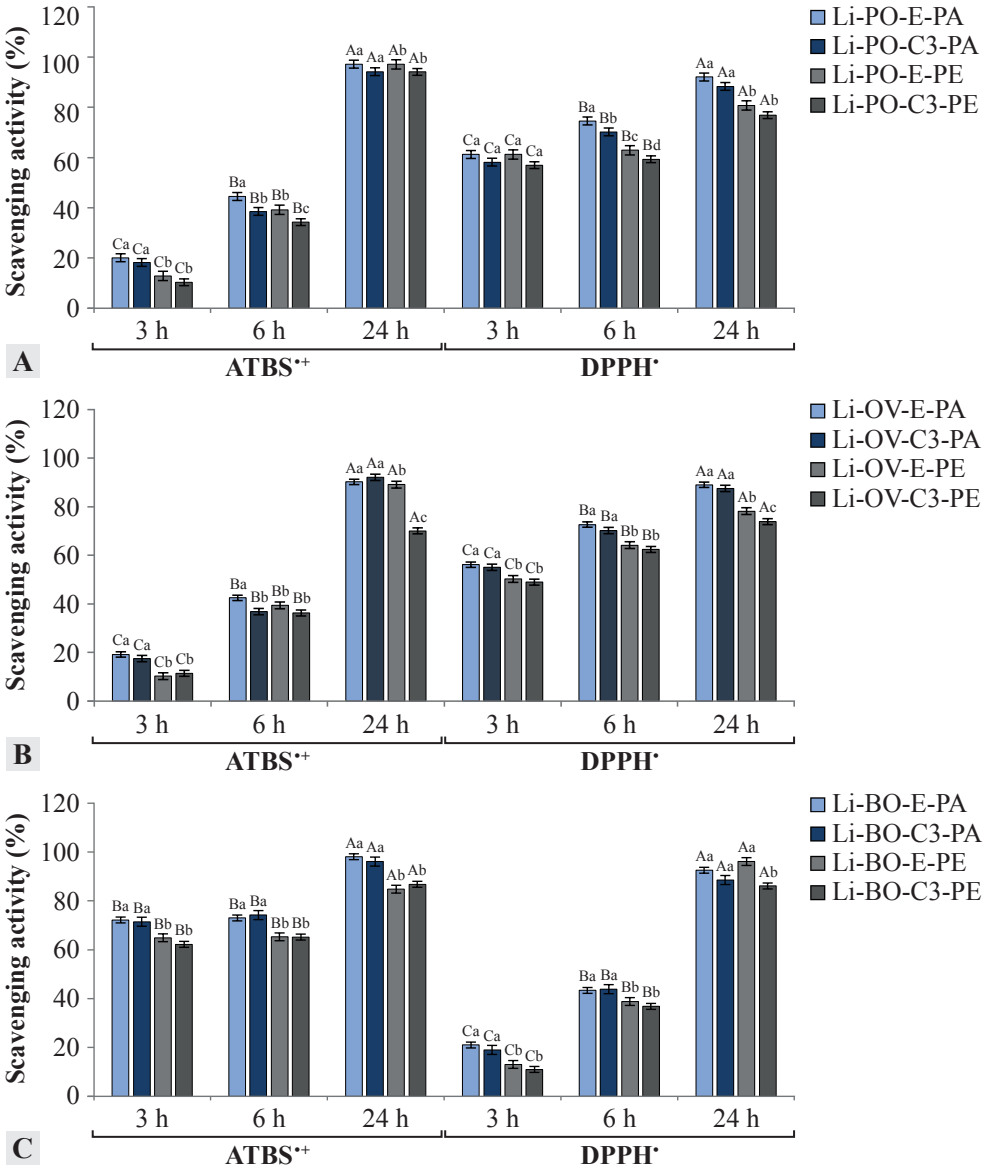


Fig. 3.5.1. *ABTS•+ and DPPH• antioxidant activity of liver hydrolysates produced using pepsin and papain at different hydrolysis times*

(A) Porcine liver; **(B)** Ovine liver; **(C)** Bovine liver. Values are presented as mean ± standard deviation (SD). Different uppercase letters (A–C) indicate statistically significant differences between hydrolysis times for the same enzyme and offal group, while different lowercase letters (a–d) indicate statistically significant differences between enzymes and offal groups at the same hydrolysis time. Statistical significance was determined by three-way analysis of variance (offal group × enzyme × time) ($p < 0.001$). Li – liver; BO – bovine; PO – porcine; OV – ovine; PA – papain; PE – pepsin; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009.

A similar time-dependent trend was observed in the DPPH$^{\bullet}$ assay. After 3 h, radical scavenging activity reached 56.15 ± 1.10% (E) and 55.09 ± 1.67% (C3) in papain-treated liver hydrolysates, and 50.28 ± 1.88% (E) and 48.98 ± 1.22% (C3) in pepsin-treated liver hydrolysates. After 6 h, the activity increased to 72.69 ± 1.67% (E) and 70.22 ± 1.31% (C3) in papain-treated liver hydrolysates, and to 64.22 ± 1.82% (E) and 62.43 ± 1.85% (C3) in pepsin-treated liver hydrolysates. The highest DPPH$^{\bullet}$ activity was observed after 24 h of hydrolysis, reaching 89.06 ± 2.23% (E) and 87.56 ± 1.14% (C3) in papain-treated liver hydrolysates, and 78.22 ± 1.13% (E) and 73.88 ± 0.85% (C3) in pepsin-treated liver hydrolysates.

The antioxidant activity of bovine liver hydrolysates was evaluated using ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ assays (Fig. 3.5.1 C). In the ABTS$^{\bullet+}$ assay, radical scavenging activity after papain treatment was already relatively high after 3 h and did not differ significantly from the values obtained after 6 h. A statistically significant increase was observed only after 24 h of hydrolysis ($p < 0.001$), when antioxidant activity reached 98.10 ± 0.30% in edible offal and 96.10 ± 0.30% in Category 3 raw material. A similar trend was observed in pepsin-treated liver hydrolysates, where no significant differences were found between 3 h and 6 h ($p > 0.05$), while a significant increase in activity was recorded after 24 h of hydrolysis ($p < 0.001$). In the DPPH$^{\bullet}$ assay, a statistically significant increase in radical scavenging activity was also observed after 24 h of hydrolysis ($p < 0.001$). The highest antioxidant activity values were determined in liver hydrolysates treated with papain, reaching 96.12 ± 1.11% (E) and 86.12 ± 1.36% (C3).

3.6. Antimicrobial activity of liver hydrolysates

The results of the antimicrobial activity of liver hydrolysates against five bacterial strains – *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 13932, *Salmonella Typhimurium* ATCC 14028, and *Bacillus cereus* ATCC 11778 – after 3, 6, and 24 h of hydrolysis are presented in Table 3.6.1.

The strongest antibacterial activity was observed in porcine liver (PO) hydrolysates produced with papain after 24 h of hydrolysis. The sample Li-PO-E-PA showed strong inhibition against *L. monocytogenes* (22.0 ± 0.2 mm) and *B. cereus* (19.5 ± 0.1 mm), as well as moderate inhibition of *S. Typhimurium* (12.5 ± 0.2 mm). A similar pattern was observed in the Li-PO-C3-PA sample, although the inhibition zones were slightly smaller. After 24 h of hydrolysis, the inhibition zones increased significantly compared with shorter hydrolysis times ($p < 0.05$), indicating a time-dependent increase in antibacterial activity.

Table 3.6.1. *Determination of the antimicrobial activity of liver hydrolysates obtained using pepsin and papain*

Sample code	Time (h)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*L. monocytogenes* ATCC 13932	*S. Typhimurium* ATCC 14028	*B. cereus* ATCC 11778
Li-PO-E-PA	3	N.D.	BS	10.2 ± 0.1^{b}	8.5 ± 0.2^{b}	8.0 ± 0.1^{c}
Li-PO-C3-PA		N.D.	BS	9.8 ± 0.1^{b}	9.2 ± 0.1^{b}	8.2 ± 0.1^{c}
Li-PO-E-PA	6	N.D.	BS	10.7 ± 0.1^{b}	8.6 ± 0.2^{b}	8.5 ± 0.2^{b}
Li-PO-C3-PA		N.D.	BS	9.5 ± 0.2^{b}	8.0 ± 0.1^{c}	10.2 ± 0.2^{b}
Li-PO-E-PA	24	N.D.	BS	22.0 ± 0.2^{a}	12.5 ± 0.2^{a}	19.5 ± 0.1^{a}
Li-PO-C3-PA		N.D.	BS	16.5 ± 0.2^{a}	10.0 ± 0.1^{a}	15.6 ± 0.2^{a}
Li-PO-E-PE	3	N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-E-PE	6	N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-E-PE	24	N.D.	BS	15.8 ± 0.1	N.D.	14.2 ± 0.1
Li-PO-C3-PE		N.D.	BS	15.0 ± 0.1	N.D.	13.0 ± 0.1
Li-OV-E-PA	3	N.D.	BS	10.2 ± 0.1^{c}	10.0 ± 0.1^{a}	N.D.
Li-OV-C3-PA		N.D.	BS	10.0 ± 0.1^{b}	9.2 ± 0.1^{b}	N.D.
Li-OV-E-PA	6	N.D.	BS	11.7 ± 0.1^{b}	10.0 ± 0.1^{a}	N.D.
Li-OV-C3-PA		N.D.	BS	10.0 ± 0.1^{b}	10.0 ± 0.1^{a}	N.D.
Li-OV-E-PA	24	N.D.	BS	17.5 ± 0.1^{a}	10.0 ± 0.1^{a}	N.D.
Li-OV-C3-PA		N.D.	BS	17.0 ± 0.1^{a}	10.0 ± 0.1^{a}	N.D.

Table 3.6.1. *Continued*

Sample code	Time (h)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*L. monocytogenes* ATCC 13932	*S. Typhimurium* ATCC 14028	*B. cereus* ATCC 11778
Li-OV-E-PE	3	N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-E-PE	6	N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-E-PE	24	N.D.	BS	N.D.	N.D.	N.D.
Li-OV-C3-PE		N.D.	BS	N.D.	N.D.	N.D.
Li-BO-E-PA	3	N.D.	BS	9.8 ± 0.1^{c}	10.0 ± 0.1^{a}	N.D.
Li-BO-C3-PA		N.D.	BS	9.0 ± 0.1^{c}	9.2 ± 0.1^{b}	N.D.
Li-BO-E-PA	6	N.D.	BS	11.7 ± 0.2^{b}	10.0 ± 0.1^{a}	N.D.
Li-BO-C3-PA		N.D.	BS	11.2 ± 0.2^{b}	10.0 ± 0.1^{a}	N.D.
Li-BO-E-PA	24	N.D.	BS	17.5 ± 0.2^{a}	10.0 ± 0.2^{a}	N.D.
Li-BO-C3-PA		N.D.	BS	17.8 ± 0.2^{a}	10.0 ± 0.1^{a}	N.D.
Li-BO-E-PE	3	N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-E-PE	6	N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-E-PE	24	N.D.	BS	N.D.	N.D.	N.D.
Li-BO-C3-PE		N.D.	BS	N.D.	N.D.	N.D.

Different superscript letters ($^{a–c}$) within the same microorganism and enzyme denote statistically significant differences between hydrolysis times, as determined by Tukey's HSD test ($p < 0.05$). Li – liver; BO – bovine; PO – porcine; OV – ovine; PA – papain; PE – pepsin; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009). N.D. – no inhibition was detected; BS – bacteriostatic effect.

In contrast, porcine hydrolysates produced with pepsin showed no inhibition after 3 and 6 h of hydrolysis. Limited antibacterial activity was detected after 24 h, where Li-PO-E-PE inhibited *L. monocytogenes* (15.8 ± 0.1 mm) and *B. cereus* (14.2 ± 0.1 mm), while Li-PO-C3-PE inhibited *L. monocytegenes* (15.0 ± 0.1 mm) and *B. cereus* (13.0 ± 0.1 mm). Bovine liver hydrolysates produced with papain for 24 h also demonstrated antibacterial activity, particularly against *L. monocytogenes* (17.5 ± 0.2 mm) and *S. Typhimurium* (10.0 ± 0.1 mm), with significant differences compared with shorter hydrolysis times ($p < 0.05$). The minimum inhibitory concentration (MIC) against *Listeria monocytogenes* in liver hydrolysates was 1.25 µg/mL, indicating strong antimicrobial activity.

Considering the established antimicrobial and antioxidant properties, porcine liver hydrolysates were selected for further studies, as they showed higher activity than other tested samples.

3.7. Peptide identification in porcine liver hydrolysates

After 24 h of hydrolysis, the peptides identified in porcine liver hydrolysates are presented in Table 3.7.1. These peptides were obtained using two different proteolytic enzymes, pepsin (P) and papain (PA). All peptides were identified in hydrolysates derived from liver (LI), edible offal and Category 3 by-products. The antioxidant activity of each hydrolysate was assessed using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radical scavenging assays, and correlations between antioxidant activity and the peak areas of peptides identified by LC–MS/MS and quantified by SWATH-MS were evaluated.

Peptide sequence analysis revealed that peptides identified in edible offal hydrolysates generally exhibited stronger positive linear correlations with antioxidant activity compared to peptides identified in hydrolysates obtained from Category 3 meat by-products. The highest correlations with $DPPH^{\bullet}$ antioxidant activity were observed for the peptides MPVRVVSPDN ($r = 0.896$), VGNLSLGAVQMIGD ($r = 0.884$), and RSGTPKPSTPTPTP ($r = 0.865$). Strong positive linear correlations were also identified for the peptides YNGEEPKETLPFPIID ($r = 0.815$), derived from peroxiredoxin-6 (PRDX6), a protein involved in antioxidant defence, and LLRNHPEPT ($r = 0.797$), derived from aldehyde oxidase 1 (AOX1), which is associated with oxidative reactions.

Table 3.7.1. *Peptides identified in porcine liver hydrolysates after 24 h of hydrolysis and their correlation with antioxidant activity (DPPH• and ABTS•+)*

Peptide sequence	Protein	Biological function of protein	Gene	Enzyme	r (DPPH• E / C3)	r (ABTS•+ E/C3)	Molecular weight (kDa)
YNGEEPKETLPFPIID	Peroxiredoxin 6	Reactive oxygen species neutralization	PRDX6	P	0.815*/0.675*	0.718*/0.575*	1.862
FDMAAVLQQVL	TPR nucleoprotein	Regulation of nucleocytoplasmic transport	TPR	P	0.675*/0.221	0.886*/0.664*	1.234
LIRLDST	DENN domain-containing protein 4C	Rab GTPase activation and vesicular transport	DENND4C	P	0.332/N.D.	0.441/N.D.	0.817
AELIFVGMT	Zinc finger protein	DNA binding and transcription regulation	ZNF280B	P	0.567*/N.D.	0.455/N.D.	0.980
FLAVSDSETRS	Zinc finger protein	DNA binding and transcription regulation	ZNF280B	P	0.689*/0.568*	0.785*/0.768*	1.211
RSGTPKPSTPTPTP	SPT20 homolog, SAGA complex component	Regulation of gene expression	SUPT20H	P	0.865*/N.D.	0.765*/N.D.	1.424
IIDAPGHRDF	Eukaryotic translation elongation factor 1 alpha 1	Aminoacyl-tRNA transport during protein synthesis	EEF1A1	P	0.234/N.D.	0.123/N.D.	1.140

Table 3.7.1. *Continued*

Peptide sequence	Protein	Biological function of protein	Gene	Enzyme	r (DPPH· E / C3)	r ($ABTS^{\cdot+}$ E/C3)	Molecular weight (kDa)
SDYPPLGRF	Eukaryotic translation elongation factor 1 alpha 1	Aminoacyl-tRNA transport during protein synthesis	EEF1A1	P	0.443/N.D.	0.249/N.D.	1.051
VGNLSLGAVQMIGD	Amine oxidase	Oxidative degradation of biogenic amines	LOC100525099	P	0.884*/0.765*	0.887*/0.675*	1.374
LPWGSRGA	Myosin XVB	Actin-dependent intracellular transport	MYO15B	P	0.768*/0.674*	0.889*/0.765*	0.843
DMHTKPWVR	Betaine–homocysteine S-methyltransferase 1	Remethylation of homocysteine to methionine	BHMT	P	0.696*/0.587*	0.567*/0.678*	1.169
LDMHTKPWVR	Betaine–homocysteine S-methyltransferase 1	Remethylation of homocysteine to methionine	BHMT	P	0.765*/0.675*	0.567*/0.768*	1.283
QAFDIGTLR	Cadherin 9	Calcium-dependent cell adhesion	CDH9	PA	0.678*/N.D.	0.675*/N.D.	1.020
MPRRTH	ATG2A autophagy-related protein	Regulation of autophagosome formation	ATG2A	PA	0.598*/0.678*	0.686*/0.786*	0.797

Table 3.7.1. *Continued*

Peptide sequence	Protein	Biological function of protein	Gene	Enzyme	r (DPPH· E / C3)	r (ABTS·+ E/C3)	Molecular weight (kDa)
LERGEDMLITGGR	Aldehyde oxidase 1	Oxidation of aldehydes and xenobiotics	AOX1	PA	0.765*/N.D.	0.678*/N.D.	1.447
LLRNHPEPT	Aldehyde oxidase 1	Oxidation of aldehydes and xenobiotics	AOX1	PA	0.797*/N.D	0.885*/N.D.	1.076
LVTASMPGTKT	Unc-80 homolog, NALCN channel complex subunit	Ion channel regulation	UNC80	PA	0.495/N.D.	0.396/N.D.	1.105
MPVRVVSPDN	Teneurin trans-membrane protein 3	Formation of neuronal connections	TENM3	PA	0.896*/0.881*	0.596*/0.662*	1.113
MQQLRVQLEKMFEA	Unc-13 homolog B	Regulation of synaptic vesicle exocytosis	UNC13B	PA	0.556*/0.443	0.695*/0.675*	1.751

Peptides demonstrating at least one statistically significant association with antioxidant activity as determined by Pearson. Peptides demonstrating at least one statistically significant association with antioxidant activity as determined by Pearson correlation analysis ($p < 0.05$). P – pepsin; PA – papain; E – edible offal; C3 – Category 3 animal by-products according to Regulation (EC) No 1069/2009. N.D. – not detected.

The highest correlations with $ABTS^{\bullet+}$ antioxidant activity were observed for the peptides LPWGSRGA (r = 0.889), VGNLSLGAVQMIGD (r = 0.887), FDMAAVLQQVL (r = 0.886), and LLRNHPEPT (r = 0.885), indicating their strong free radical scavenging potential. Moderate positive linear correlations were observed for the peptides FLAVSDSETRS, RSGTPKPSTPTPTP, MPRRTH, and MQQLRVQLEKMFEA.

Most identified peptides were characterised by low molecular weight (< 2 kDa), which may be associated with enhanced antioxidant activity and more effective free radical scavenging capacity. Some peptides were identified only in edible offal hydrolysates, whereas their antioxidant activity was not detected in hydrolysates obtained from Category 3 meat by-products.

Despite the observed antibacterial activity of the hydrolysates, none of the identified peptides could be unambiguously assigned to peptides with known antimicrobial properties based on the databases. Therefore, correlation analyses were limited to the relationships between the identified antioxidant peptides and the antioxidant activity of the hydrolysates.

Although antibacterial activity was observed in the hydrolysates, none of the peptides identified by LC–MS/MS were assigned antibacterial properties based on the bioactive peptide databases used. Therefore, the correlation analysis was limited to evaluating the relationships between peptides identified as possessing antioxidant properties and the antioxidant activity of the hydrolysates.

Liver hydrolysates from edible offal produced using papain were selected for gummy production based on their higher antioxidant activity, more bioactive peptide profile, and greater functional potential compared with pepsin-derived hydrolysates and hydrolysates produced from Category 3 animal by-products.

3.8. Microbiological quality of gummy candies with liver hydrolysates during storage

The microbiological quality of gummy candy samples prepared with porcine liver hydrolysates and agar as the gelling agent was evaluated over a 21-day storage period (Table 3.8.1). No aerobic mesophilic bacteria were detected in any of the samples throughout storage, except for the control sample (GC), in which a total viable count (TVC) of 2.55 $\log_{10}$ CFU/g was observed on day 21. In contrast, all hydrolysate-enriched samples (GC5LPa3Ag, GC5LPa6Ag, and GC5LPa24Ag) maintained TVC values below the detection limit (< 1.0 $\log_{10}$ CFU/g) during the entire storage period. Moulds and yeasts were not detected in any sample at any storage period, with all values remaining below the detection limit (< 1.0 $\log_{10}$ CFU/g).

Table 3.8.1. *Microbiological quality of gummy candy samples using liver hydrolysates and gel-forming agents throughout a 21-day storage period*

Sample code	TVC			Moulds and Yeasts		
	Day 1	Day 7	Day 21	Day 1	Day 7	Day 21
	(log_{10} CFU/g)			(log_{10} CFU/g)		
GC	< 1.0	< 1.0	2.55	< 1.0	< 1.0	< 1.0
GC5LPa3Ag	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
GC5LPa6Ag	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
GC5Pa24Ag	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

GC – gummy candy; 5.0 – content of hydrolysates (g); 3, 6, and 24 h – hydrolysis time; Pa – papain hydrolysis; Ag – agar.

3.9. Nutritional composition of gummy candy with liver hydrolysates

The nutritional composition of the gummy candies is presented in Table 3.9.1. The incorporation of porcine liver hydrolysates had a significant impact on the nutritional profile of the gummies. Protein content differed significantly between the control and hydrolysate-enriched samples ($p < 0.05$). The lowest protein content was observed in the control sample (GC) (0.04 ± 0.01 g/100 g), whereas the hydrolysate-enriched formulations contained markedly higher protein levels, ranging from 4.48 ± 0.02 to 4.56 ± 0.03 g/100 g.

Ash content also increased significantly following the addition of porcine liver hydrolysates ($p < 0.05$). The control sample exhibited the lowest ash content (0.16 ± 0.02 g/100 g), while the enriched samples contained between 0.44 ± 0.03 and 0.48 ± 0.02 g/100 g.

Carbohydrate content differed significantly between the GC and hydrolysate-enriched formulations ($p < 0.05$). The GC contained the lowest carbohydrate content (14.57 ± 0.03 g/100 g), whereas all hydrolysate-containing samples showed significantly higher values, ranging from 19.94 ± 0.02 to 20.42 ± 0.03 g/100 g. However, no significant differences in carbohydrate content were detected among the hydrolysate-enriched formulations. No statistically significant differences in fat content were observed among the samples ($p > 0.05$).

Table 3.9.1. *Composition of gummy candy samples using liver hydrolysates and gel-forming agents (g/100 g)*

Sample code	Protein, g/100 g	Fat, g/100 g	Ash, g/100 g	Carbohydrates, g/100 g
GC	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.16 ± 0.02^{a}	14.57 ± 0.03^{b}
GC5LPa3Ag	4.48 ± 0.02^{b}	0.04 ± 0.01^{a}	0.44 ± 0.03^{b}	19.94 ± 0.02^{a}
GC5LPa6Ag	4.53 ± 0.0^{b}	0.03 ± 0.01^{a}	0.46 ± 0.03^{b}	20.18 ± 0.02^{a}
GC5LPa24Ag	4.56 ± 0.03^{b}	0.04 ± 0.01^{a}	0.48 ± 0.02^{b}	20.42 ± 0.03^{a}

[a–c] Mean values in columns with different letters differ significantly ($p \leq 0.05$). Data are presented as mean ($n = 3$) ± standard deviation (SD). GC – gummy candies; 5.0 - hydrolysate content (g); 3, 6, and 24 h – hydrolysis time; Pa – papain hydrolysis; Ag – agar.

3.10. Antioxidant activity ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays) of gummy candies enriched with porcine liver hydrolysates

The antioxidant properties ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ free radical scavenging activity) and pH values of gummy candies prepared with porcine liver hydrolysates are presented in Table 3.10.1. The antioxidant properties ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging activity) and pH values of gummy candies prepared with porcine liver hydrolysates are presented in Table 3.10.1. The control sample (GC) exhibited the lowest pH value (3.00 ± 0.02) and the weakest antioxidant activity, as determined by the $ABTS^{\bullet+}$ (8.52 ± 0.58 μmol/g) and $DPPH^{\bullet}$ (8.62 ± 0.16%) assays. The incorporation of porcine liver hydrolysates significantly increased both pH and antioxidant activity ($p < 0.05$). Radical scavenging activity increased progressively with increasing hydrolysis duration. The $ABTS^{\bullet+}$ scavenging activity increased from 24.60 ± 0.93 μmol/g in GC5LPa3Ag to 52.67 ± 0.50 μmol/g and 67.60 ± 0.98 μmol/g in GC5LPa6Ag and GC5LPa24Ag, respectively. A similar tendency was observed for $DPPH^{\bullet}$ radical scavenging activity, which increased from 11.93 ± 0.68% in GC5LPa3Ag to 33.29 ± 1.07% and 49.14 ± 1.00% in GC5LPa6Ag and GC5LPa24Ag, respectively.

Table 3.10.1. *Antioxidant activity (ABTS$^{\bullet+}$, µmol/g; DPPH$^{\bullet}$, %) and pH of gummy candies formulated with porcine liver hydrolysates*

Sample code	pH	ATBS$^{\bullet+}$ scavenging activity	DPPH$^{\bullet}$ scavenging activity
GC	3.00 ± 0.02^{b}	8.52 ± 0.58^{d}	8.62 ± 0.16^{d}
GC5LPa3Ag	3.66 ± 0.02^{a}	24.6 ± 0.93^{c}	11.93 ± 0.68^{c}
GC5LPa6Ag	3.70 ± 0.03^{a}	52.67 ± 0.5^{b}	33.29 ± 1.07^{b}
GC5Pa24Ag	3.65 ± 0.03^{a}	67.6 ± 0.98^{a}	49.14 ± 1.0^{a}

[a–d] Mean values in columns with different letters differ significantly ($p \leq 0.05$). Data are presented as mean (n = 3) ± standard deviation (SD). GC – gummy candies; 5.0 – hydrolysate content (g); 3, 6, and 24 h – hydrolysis time; Pa – papain hydrolysis; Ag – agar.

3.11. Antibacterial activity of gummy candy enriched with porcine liver hydrolysates

The antibacterial activity of gummy candies enriched with porcine liver hydrolysates obtained using papain at different hydrolysis durations is presented in Table 3.11.1. The control sample (GC) exhibited the weakest antibacterial activity against all tested bacteria, with inhibition zone diameters ranging from 11.0 ± 0.1 mm against *S. aureus* subsp. *aureus* and *L. monocytogenes* to 16.0 ± 0.1 mm against *S. enterica* subsp. *enterica* serovar Typhimurium ($p < 0.05$). The incorporation of porcine liver hydrolysates significantly enhanced the antibacterial activity of the gummies. The sample GC5LPa3Ag, containing hydrolysate obtained after 3 h of hydrolysis, exhibited stronger inhibitory effects against *E. coli* (16.0 ± 0.1 mm) and particularly against *L. monocytogenes* (21.0 ± 0.1 mm) compared with the control sample ($p < 0.05$).

A further increase in antibacterial activity was observed with increasing hydrolysis duration. The sample GC5LPa6Ag exhibited strong inhibitory effects against all tested bacteria, with inhibition zone diameters of 29.0 ± 0.2 mm against *E. coli*, 27.0 ± 0.1 mm against *S. aureus* subsp. *aureus*, 30.0 ± 0.2 mm against *L. monocytogenes*, 19.0 ± 0.1 mm against *S. enterica* subsp. *enterica* serovar Typhimurium, and 30.0 ± 0.3 mm against *B. cereus* ($p < 0.05$).

The GC5Pa24Ag sample also exhibited pronounced antibacterial activity, with inhibition zone diameters of 29.0 ± 0.2 mm against *E. coli*, 28.0 ± 0.1 mm against *S. aureus* subsp. *aureus*, 30.5 ± 0.2 mm against *L. monocytegenes*, and 22.0 ± 0.2 mm against *S. enterica* subsp. *enterica* serovar Typhimurium. However, although the antibacterial activity against *B. cereus* (25.0 ± 0.2 mm) was lower than that observed for GC5LPa6Ag, it remained significantly higher than that of the control and GC5LPa3Ag samples ($p < 0.05$).

Table 3.11.1. *Antibacterial activity of gummy candies with porcine hydrolysates*

Sample code	Diameter of inhibition zones (mm)				
	E. coli **ATCC 25922**	***S. aureus*** **subsp. *aureus* ATCC 25923**	***L. monocytogenes*** **ATCC 13932**	***S. enterica*** **subsp. *enterica* serovar Typhimurium ATCC 14028**	***B. cereus*** **ATCC 11778**
GC	12.0 ± 0.1^{c}	11.0 ± 0.1^{b}	11.0 ± 0.1^{c}	16.0 ± 0.1^{c}	15.0 ± 0.1^{c}
GC5LPa3Ag	16.0 ± 0.1^{b}	11.0 ± 0.1^{b}	21.0 ± 0.1^{b}	16.0 ± 0.1^{c}	15.0 ± 0.1^{c}
GC5LPa6Ag	29.0 ± 0.2^{a}	27.0 ± 0.1^{a}	30.0 ± 0.2^{a}	19.0 ± 0.1^{b}	30.0 ± 0.3^{a}
GC5Pa24Ag	29.0 ± 0.2^{a}	28.0 ± 0.1^{a}	30.5 ± 0.2^{a}	22.0 ± 0.2^{a}	25.0 ± 0.2^{b}

$^{a–c}$ Mean values in columns with different letters differ significantly ($p \leq 0.05$); data are presented as mean ($n = 3$) ± standard deviation (SD); GC – gummy candy; 5.0 – hydrolysate content (g); 3, 6, and 24 h – hydrolysis time, Pa – papain hydrolysis; Ag – agar.

3.12. Physicochemical properties and overall acceptability of gummy candies enriched with liver hydrolysates

The color parameters, texture properties, and overall acceptability of gummy candies formulated with porcine liver hydrolysates are presented in Table 3.12.1. The incorporation of porcine liver hydrolysates significantly affected all colour parameters (L*, a*, b*, C*, and h) as well as texture hardness ($p < 0.05$), whereas no significant differences were observed in overall acceptability ($p > 0.05$). The control sample (GC) exhibited the highest lightness ($L^* = 39.83 \pm 0.72$) and hue angle ($h = 262.23 \pm 1.74$), while hydrolysate-enriched samples showed significantly lower L* values, indicating a darker appearance. The addition of porcine liver hydrolysates also resulted in a shift from negative to positive a* and b* values, suggesting an increase in red and yellow colour tones. The highest redness ($a^* = 0.63 \pm 0.06$), yellowness ($b^* = 6.09 \pm 0.04$), and chroma ($C^* = 6.11 \pm 0.06$) values were observed in GC5LPa3Ag, whereas GC retained the lowest values for these parameters. The hardness of the gummy candies was influenced by the hydrolysis duration of the porcine liver hydrolysates used. Although the hardness values of GC5LPa3Ag and GC5LPa6Ag did not differ significantly from that of the control sample, the sample containing the hydrolysate subjected to the longest hydrolysis duration (GC5LPa24Ag) exhibited significantly greater hardness (4.63 ± 0.15 mJ; $p < 0.05$). Despite the observed differences in colour and texture characteristics, overall acceptability scores remained similar among all samples, ranging from 7.0 ± 0.3 to 7.5 ± 0.5, with no statistically significant differences detected ($p > 0.05$).

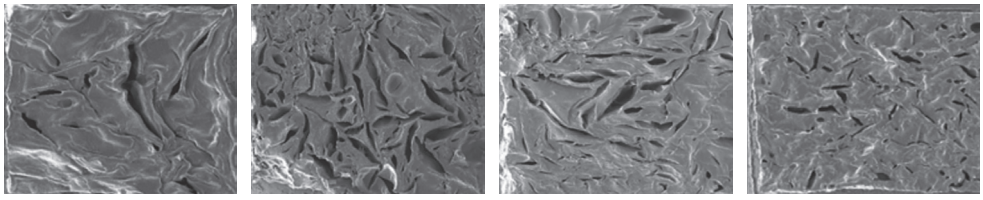
Table 3.12.1. *The color parameters, texture properties, and overall acceptability of gummy candies formulated with porcine liver hydrolysates*

Sample code	Colour coordinates, NBS					Texture hardness (mJ)	Overall acceptability
	L*	a*	b*	c*	h		
GC	39.83 ± 0.72[a]	–0.02 ± 0.54[a]	–3.03 ± 0.47[d]	3.06 ± 0.49[c]	262.23 ± 1.74[a]	3.33 ± 0.06[c]	7.5 ± 0.5[a]
GC5LPa3Ag	35.26 ± 0.42[b]	0.63 ± 0.06 [a]	6.09 ± 0.04[a]	6.11 ± 0.06[a]	84.11 ± 0.62[c]	3.73 ± 0.15[b]	7.0 ± 0.8[a]
GC5LPa6Ag	32.77 ± 0.76 [c]	0.43 ± 0.1[a]	5.01 ± 0.05[b]	5.03 ± 0.04[b]	85.33 ± 0.86[c]	3.63 ± 0.06[b]	7.0 ± 0.3[a]
GC5Pa24Ag	36.01 ± 1.49[b]	0.30 ± 0.02[a]	3.74 ± 0.12[c]	3.73 ± 0.14[c]	92.76 ± 0.57[b]	4.63 ± 0.15[a]	7.0 ± 0.5[a]

[a–c] Mean values in columns with different letters differ statistically ($p \leq 0.05$); data are presented as mean (n = 3) ± standard deviation (SD). GC – gummy candy; 5.0 – hydrolysate content (g); 3, 6, and 24 h – hydrolysis time; Pa – papain hydrolysis; Ag – agar.

3.13. Microstructural analysis of gummy candies using scanning electron microscopy (SEM)

The microstructural differences of the analyzed samples are presented in Fig. 3.13.1. The control sample (GC) exhibited a homogeneous, slightly embossed surface without a clearly defined pore structure. The samples with liver hydrolysates (GC5Pa3Ag, GC5Pa6Ag, GC5Pa24Ag) were distinguished by higher microporosity and more pronounced phase separation.



GC GC5LPa3Ag GC5LPa6Ag GC5LPa24Ag

Fig. 3.13.1. *SEM micrographs of the samples captured at 5.0 kV accelerating voltage and 1.00 k× magnification (scale bar represents 50 μm)*

GC – gummy candy; 5.0 – content of hydrolysates (g); 3, 6, and 24 h – hydrolysis time; Pa – papain hydrolysis; Ag – agar.

4. DISSCUSION

4.1. Evaluation of offal as potential raw materials for protein hydrolysate production

In the European Union, post-mortem inspection is the final stage of official control aimed at protecting public health and ensuring animal welfare by addressing risks related to zoonoses and animal diseases. In addition to its regulatory function, post-mortem inspection is a key component of veterinary public health surveillance, as it allows for the detection of pathological lesions associated with subclinical conditions that may be missed during ante-mortem inspection. This information provides valuable epidemiological insights into disease prevalence in animal populations. Previous studies have revealed a consistent pattern of distribution of pathoanatomical lesions in carcasses of slaughtered porcine. Vladimír Večerek et al. [263] reported that the overall prevalence of post-mortem lesions exceeded 10%, with the lungs, kidneys, and liver being the most commonly affected organs. Similar results were reported by Januškevičienė et al. [9], who found that pathological lesions were mainly localised in the respiratory system and liver, while renal pathologies were recorded less frequently. The results of the present study are partially consistent with these findings, as the overall prevalence of pathological lesions in porcine carcasses also exceeded 10%. However, in the present study, lesions were most frequently observed in the cardiovascular (4.83%) and digestive (4.50%) systems.

Previous post-mortem data on bovines indicate that pathological lesions are not evenly distributed among organ systems. Sebastian Ciui et al. [6] reported that pathological lesions are most frequently detected in the liver and less frequently in the lungs, heart, and kidneys. Analysis of cattle categories by other authors showed that cows, compared to heifers and bulls, had a higher incidence of pathological lesions, which is associated with higher farming intensity and increased metabolic load in dairy production systems. A similar tendency is reflected in our study. The distribution of pathological lesions in cows shows that lesions are most often found in the digestive system 33 (10.09%), less often – in the respiratory tract 18 (5.50%). Statistically significant differences between cattle categories were not found in any of the studied organ systems, which indicates a fairly even distribution of pathological lesions between cows, bulls and heifers.

Previous ovine slaughter data have shown that the liver (51.0%) and lungs (48.7%) are the most commonly affected organs in ovine during post-mortem examinations, while pathological lesions in the heart and kidneys are recorded much less frequently [69]. The main reasons for confiscation of

ovine organs reported in previous studies are liver cirrhosis (12.3%), pulmonary pneumonia (6.6%), pericarditis (0.6%) and renal nephritis (0.6%) [264]. A similar distribution was observed in this study, where the most frequently recorded pathological findings were related to the liver and accounted for 5.50% of the examined carcasses. The predominant liver lesions were fibrosis (14 cases; 2.33%) and fatty degeneration (9 cases; 1.50%). Pathological lesions in other organ systems were relatively rare: pulmonary emphysema was found in 9 carcasses (1.50%), myocardial haemorrhage in 9 animals (1.50%), renal cysts in 8 cases (1.33%), and nephritis in only 2 animals (0.33%).

Due to their high water activity, abundance of soluble nutrients, and intensive autolytic and enzymatic processes, animal-derived by-products are highly susceptible to rapid microbiological spoilage [265, 266]. These conditions create a favourable environment for the proliferation of pathogenic microorganisms, including *Salmonella* spp., *Clostridium perfringens*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7, all of which are significant causative agents of foodborne illnesses [100, 101]. In the present study, the concentrations of *E. coli*, *Salmonella* spp., and *Listeria monocytogenes* in all analyzed samples were below the detection limit. Considering that carcass contamination with pathogens such as *Salmonella* spp. and *E. coli* represents a major food safety concern, international guidelines recommend avoiding palpations and incisions during post-mortem inspection to reduce the risk of cross-contamination and subsequent dissemination of biological hazards [100]. The concentrations of coliform bacteria and *E. coli* are widely regarded as indicators of faecal contamination in food products; therefore, their low levels in this study confirm appropriate raw material handling and processing conditions [267]. Other authors have similarly reported that the prevalence of *Salmonella* spp. in organs such as liver, kidneys, heart, and lungs is very low or undetectable [268]. In the present study, *Enterobacteriaceae* concentrations were below the detection limit (< 10 CFU/g) in most samples, although low levels (2.16–2.26 $\log_{10}$ CFU/g) were detected in several liver samples. Erickson et al. [268] demonstrated that the prevalence of *Listeria monocytogenes* in certain meat by-products can be substantial, with up to 33% of beef by-product samples testing positive and prevalence in lung samples reaching 50%. These findings are generally attributed to cross-contamination during slaughter and primary processing, as well as insufficient control of hygiene practices. The results of this study also showed that the highest TAMC values were observed in lung and heart samples, particularly those from porcine and bovine, with counts ranging from 4.4 to 4.65 $\log_{10}$ CFU/g. In contrast, TAMC values in ovine samples were significantly lower. Comparable TAMC levels were reported by Vanderlinde et al. [43], who found that total aerobic counts in bovine, ovine, and goat by-products ranged from 2.3 to 4.6 $\log_{10}$ CFU/g.

Microbial contamination of by-products can increase at various stages of slaughter and post-mortem inspection, particularly during evisceration, hide removal, incisions, and palpations, where tissue disruption, increased surface exposure, and inadequate hygiene practices facilitate microbial transfer and growth [43].

Due to their persistence and lipophilicity, organochlorine pesticides (OCPs) tend to accumulate in fatty tissues and edible offal and are still detected in bovine by-products, with concentrations in the liver and kidneys typically ranging from 0.006 to 0.035 mg/kg, depending on the region and the level of environmental contamination [269]. No residues of organochlorine pesticides (OCPs) were detected in our samples – all analyzed compounds, including aldrin, dieldrin, heptachlor epoxide, chlordanes, and DDT with its metabolites, were below the limit of detection (LOD). The highest levels are typically found in the kidneys, where concentrations of DDT, DDE, or HCH may reach 0.028–0.031 mg/kg, confirming the tendency of these organs to accumulate lipophilic pesticides, as reported in other authors' studies [270].

Heavy metals enter animal tissues when grazing in contaminated soils and through feed and water affected by environmental factors [271]. The liver and kidneys are at particularly high risk of bioaccumulation, as these organs accumulate toxic substances more intensively than other tissues. [272]. In our study, Hg and Pb concentrations in all offal samples were below the limit of detection, while Cd was detected only in the liver (0.06–0.09 mg/kg) and kidneys (0.41–0.58 mg/kg), with the highest levels in the kidneys ($p < 0.05$); however, all detected concentrations did not exceed the maximum permitted levels. Previous studies have shown that elevated Hg concentrations exceeding the established permissible limits were found in 57.1% of liver samples and 40% of kidney samples [271]. However, these results differ from the studies conducted by Hashemi [273] and Unguryanu et al. [274], in which Hg concentrations in all examined liver and kidney samples did not exceed the recommended maximum limits. In earlier studies, Cd concentrations ranged from 0.088 to 4.493 mg/kg in kidneys and from 0.016 to 0.206 mg/kg in livers. The permissible Cd limit was exceeded in 15% of kidney samples, while no exceedances were detected in liver samples [275].

To expand the scope of application of offal in the food industry, it is necessary to assess its nutritional value and safety. Therefore, in the first stage of this study, the nutritional composition and safety indicators of selected edible offal and Category 3 animal by-products were investigated.

Protein composition analysis showed that liver samples had the highest protein content (16.3–19.3 ± 0.01%), while heart samples had a relatively high protein content (15.4 ± 0.01–17.2 ± 0.02%), depending on the animal

species. These data are consistent with previous studies showing that liver is one of the most protein-rich animal by-products. According to Wioletta Biel et al. [48], the protein concentration in the liver of bovine and ovine is about 20.30%, which confirms the high nutritional value of this organ. Similar trends were observed in other studies, where porcine liver had the highest protein content (20.98%), followed by heart (17.62%), and lung had the lowest protein concentration (16.60%) [45]. The observed differences in protein concentration between offal types and animal species may be attributed to genetic factors, animal age and feeding regime, which are known to influence tissue composition [56]. Animal-sourced foods, such as meats, poultry, eggs, milk, and fish are nutrient-dense foods that are rich sources of protein, essential amino acids, and micronutrients that can be challenging to obtain solely through plant-based foods. Animal-sourced protein foods provide crucial nutrients that support the growth and development in children, maintenance of muscle mass and function in adults, gain in muscle mass and strength in exercising individuals, and mitigation of sarcopenia in the elderly. The 2020–2025 Dietary Guidelines for Americans have identified the important role of animal-sourced foods in the diet at every stage of life. Animal-sourced foods are consumed worldwide and contribute to global food security [292] [56].

Most offal is relatively low in fat. The highest fat content was determined in porcine heart samples (8.4 ± 0.02%) and ovine liver (5.2 ± 0.01%). This indicates a significant influence of organ type and animal species on lipid distribution. These results are consistent with previous studies, which reported that the kidney generally has the lowest fat content (about 3%), the heart has an average fat content of 3% to 7%, and the liver has a low to moderate fat content, usually not exceeding 5%, depending on the animal species [276]. The biological value of by-products is high due to the similarity of the essential amino acid profile to muscle proteins, and their stability during heat treatment is associated with the absence of reducing sugars [54,57]. Animal proteins are completely characterised by the presence of essential amino acids in balanced proportions that meet human physiological needs; they are classified as valuable proteins [277]. In addition, the stability of these amino acids during heat treatment can be attributed to the absence of reducing sugars, which limits their participation in the Maillard reaction [54]. In our study, the concentrations of essential amino acids varied greatly depending on the type of organ, which was found to be the main factor determining the distribution of all essential amino acids analysed. The proteins contained in by-products have a very high biological value, as essential amino acids make up a significant part of the total amino acid content (about 45–50%). Their highest concentration was found in the liver,

while in the heart, kidneys and lungs this proportion was somewhat lower, but remained sufficient to ensure complete protein nutrition. The highest total essential amino acid concentrations were found in liver samples, which consistently contained higher levels of essential amino acids, especially leucine, valine, lysine, and isoleucine, compared to organs from all animals analysed. Of all the essential amino acids analysed, leucine was the most abundant in all by-products. Other authors emphasise that the differences in the composition and amount of amino acids are related to the differences in the protein composition of the by-products. Previous studies have shown that the highest concentrations of essential amino acids, including threonine, valine, isoleucine, leucine, phenylalanine, lysine, and histidine, were found in porcine by-products, especially liver, where essential amino acids account for almost 50% of the total amino acid content [45, 56].

The fatty acid composition of animal by-products varies greatly due to species-specific metabolic characteristics and differences in the biochemical composition of individual organs [57]. Our study also found that the fatty acid composition and lipid quality indicators significantly depended on the animal species, organ type and offal group ($p < 0.05$). The most favorable lipid profile was characterized porcine liver and bovine kidney due to higher PUFA content, more favorable PUFA/SFA ratio and lower atherogenicity and thrombogenicity indices. Heart, kidney and liver are characterized by higher levels of polyunsaturated fatty acids (PUFA) and omega-6 fatty acids [278, 279]. Liver tissue actively participates in lipid metabolism and is the main site of fatty acid synthesis, therefore it can accumulate higher amounts of polyunsaturated fatty acids [280]. Florek et al. [281] was determined high PUFA content and a favorable PUFA/SFA ratio in the kidney, which is consistent with the results of this study. Bessa et al. [282] and De Smet et al. [283] emphasized that organ tissues differ significantly in lipid metabolism, with the kidney exhibiting a greater capacity to accumulate PUFA due to its physiological function and lower lipogenic activity. Category 3 (C3) animal by-products differed from edible offal (E) in a less favorable fatty acid profile, characterized by a higher proportion of saturated fatty acids (SFA) and a higher omega-6/omega-3 ratio. The observed differences between edible offal (E) and Category 3 (C3) animal by-products may be attributed to species-specific lipid metabolism characteristics, different feeding systems, and the structural and biochemical composition of the organ tissues themselves [46, 284]. These factors lead to different rates of fatty acid synthesis, accumulation, and modification, which may result in significant differences in fatty acid profiles between the E and C3 groups.

E and C3 meet established microbiological and chemical safety criteria and can be valuable sources of raw materials for the production of biologically active components.

4.2. Effect of enzymatic hydrolysis conditions on protein hydrolysis efficiency

Protein hydrolysates can be obtained by enzymatic, chemical and microbial methods, but enzymatic hydrolysis remains the most reliable method in the food and pharmaceutical fields to produce bioactive peptides [285]. In the present study, enzymatic hydrolysis was employed, and the choice of proteolytic enzymes was driven by their cleavage specificity and their reported potential to generate bioactive peptides. Proteolytic enzymes used for protein hydrolysis are usually obtained from animal (e.g., pepsin), plant (e.g., papain), and microbial sources (e.g., flavourzyme) [286]. Enzymatic protein hydrolysis using the pepsin enzyme is associated with antioxidant activity and enhanced antihypertensive potential of hydrolysates. Meanwhile, plant-derived proteases such as papain and bromelain are considered biocatalysts that promote the production of bioactive peptides with antimicrobial and antihypertensive properties [287]. Flavourzyme exhibits both endopeptidase and exopeptidase activities, allowing protein hydrolysis at internal sites and at the amino- and carboxyl-terminal ends, thereby improving the sensory properties of the resulting hydrolysates [168].

The degree of hydrolysis (DH) is considered an important indicator of enzymatic hydrolysis of proteins, as it quantitatively expresses the extent of peptide bond cleavage and allows assessing the progress of the enzymatic reaction and the efficiency of proteolytic enzymes [288]. Therefore, the degree of hydrolysis (DH) was selected as the primary criterion for enzyme evaluation in the present study. DH was assessed using three proteolytic enzymes, papain, pepsin, and flavourzyme, at different hydrolysis times (3, 6, and 24 h) to determine their efficiency and suitability for further analysis.

The degree of hydrolysis (DH) observed in this study showed a clear dependence on enzyme treatment, hydrolysis time and their interaction ($p < 0.001$) for all liver substrates. In porcine, ovine and bovine liver proteins, DH values gradually increased with hydrolysis time, with the highest values consistently recorded after 24 h. The decrease in protein content after prolonged hydrolysis may be attributed to enhanced proteolytic activity with longer reaction times [289]. Comparable findings were also described by Verma et al., who observed that DH of porcine liver proteins increased with hydrolysis time when treated with alcalase, trypsin, and papain [290]. The influence of hydrolysis time on DH values was discussed by Zhang et al.

[291] in their study of the hydrolysis of minced chicken carcass proteins, a consistent increase in DH was observed throughout the reaction period. The rapid increase in DH, usually observed in the initial stages of hydrolysis, is usually attributed to the cleavage of easily accessible peptide bonds.

Protease selection is a very important factor affecting hydrolysis efficiency, as different enzymes exhibit different catalytic specificities [292]. In this study, enzyme-related trends were clearly observed, and significant enzyme-dependent differences were found at each hydrolysis time point ($p < 0.001$). Papain treatment resulted in the highest DH values for all substrates, while flavorzyme treatment consistently showed the lowest values, regardless of hydrolysis time ($p < 0.001$). Papain exhibits broad substrate specificity, high catalytic activity, and stability over a wide pH range [293]. Therefore, the higher hydrolysis efficiency observed in this study can be attributed to these properties, as papain can cleave a variety of peptide bonds, promoting extensive proteolysis. The high hydrolysis capacity of papain towards a variety of protein substrates explains its efficiency in generating hydrolysates with increased DH values [294].

In contrast, hydrolysates obtained with flavorzyme, which has both endopeptidase and exopeptidase activities, preferentially promote peptide shortening rather than extensive protein backbone cleavage due to the dominant exopeptidase activity of flavorzyme, which may explain the observed lower DH values [295]. Pepsin-mediated hydrolysis resulted in intermediate DH values compared to the other enzymes used. This trend was consistently observed at all hydrolysis times. The moderate hydrolysis efficiency of pepsin can be attributed to its substrate specificity, as pepsin selectively cleaves peptide bonds, mainly at hydrophobic amino acid regions. This selective mechanism of action results in less protein degradation than with broad-specificity proteases such as papain, but more efficient than with enzyme preparations with dominant exopeptidase activity such as flavorzyme.

Our findings are consistent with those of Jin et al. [296], where hydrolysis using alcalase, trypsin and papain resulted in significantly higher DH values compared to flavorzyme. A similar trend was observed in the study by Czelej et al. [297], where pepsin-mediated hydrolysis was associated with intermediate DH values compared to trypsin and papain, suggesting a more selective nature of the enzyme degradation. Enzymatic hydrolysis is a stable protein degradation method due to its safety, specificity, and efficiency in producing bioactive peptides. In this study, papain exhibited the highest hydrolysis efficiency, and longer hydrolysis times increased the degree of hydrolysis.

4.3. Bioactive properties of liver hydrolysates

Enzymatic hydrolysis using proteases can enhance the biological activity of liver hydrolysates by generating bioactive peptides with antioxidant properties [298]. Proteins and peptides derived from meat and edible by-products have been reported to exhibit significant antioxidant activity due to their ability to neutralize free radicals and chelate pro-oxidant metals [183]. For example, some studies have shown that dry-aged ham samples have a higher antioxidant capacity than fish (sardines and hake) or plant-based products [299] . In addition, meat and offal contain natural antioxidants that can protect them from oxidative damage. The effectiveness of these compounds may be influenced by the genetic characteristics of the animals. It has also been found that the antioxidant activity may be determined by the conditions of animal husbandry on the farm and the meat processing technology. All these factors together influence the oxidative stability of meat and reveal the complex nature of antioxidant activity in meat products and offal [300].

In the present study, the antioxidant activity of liver hydrolysates was significantly influenced by enzyme type, hydrolysis time, and their interaction ($p < 0.001$). Radical scavenging activity increased progressively with increasing hydrolysis time and reached the highest values after 24 h of hydrolysis. Papain-treated hydrolysates generally exhibited significantly higher $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging activity compared to pepsin-treated samples ($p < 0.001$).

The influence of hydrolysis time was also analyzed by Bah et al. [300], who studied the antioxidant properties of porcine erythrocyte hydrolysates obtained using papain, and their results showed that the antioxidant activity of the hydrolysates increased with increasing hydrolysis time.

Similar trends were found by Paula Borrajo et al. [205], who noted that the hydrolysis time significantly affects the $ABTS^{\bullet+}$ radical scavenging activity. The results of the study showed that hydrolysates hydrolyzed with papain and alcalase exhibited high antioxidant activity, and this activity increased with increasing hydrolysis time, indicating a positive relationship between the hydrolysis time and the ability of the hydrolysates to neutralize free radicals. Damgaard et al. [301] also reported that liver protein hydrolysates may exhibit significant $DPPH^{\bullet}$ radical scavenging activity. It was found that papain hydrolysates exhibited higher $DPPH^{\bullet}$ radical scavenging activity compared to hydrolysates prepared using pepsin, neutral protease and trypsin.

The size of the peptides formed can determine the antimicrobial properties of hydrolysates, the molecular weight of the mixture and the structural configuration, which directly affect their ability to interact with microorganisms, for example, to damage cell membranes or disrupt metabolic

processes [302, 303]. Kim and Wijesekara indicated that certain molecules exhibit optimal antimicrobial activity at a specific size, while peptides that are too long or too short may have reduced efficacy [302] . Also important are the properties of peptides, such as hydrophilicity, lipophilicity and charge, which, together with molecular size, determine their selectivity and efficacy against different bacterial or fungal species [304]. Short peptides can diffuse rapidly without sufficiently damaging cell membranes, while longer peptides often have a stronger effect, but their absorption or stability may be limited [305]. In the present study, papain hydrolysis resulted in higher antimicrobial activity, which may be associated with the ability of this enzyme to generate low-molecular-weight peptides with enhanced biological activity [293].

Our results show that antimicrobial activity was significantly influenced by enzyme selection and hydrolysis time. The highest antimicrobial activity was observed after 24 h of papain hydrolysis ($p < 0.05$). The duration of hydrolysis affects the size distribution of peptides, and lower molecular weight fractions are often associated with increased antimicrobial activity [306]. The highest inhibitory effect against *Listeria monocytogenes* (22.0 ± 0.2 mm) and *Bacillus cereus* (19.5 ± 0.1 mm) was determined using porcine liver as a substrate (Li-PO-E-PA) in a sample after 24 h of hydrolysis. These antimicrobial properties may be attributed to the efficient interaction of peptides with bacterial membrane structures. Both species are Gram-positive bacteria characterized by a thick peptidoglycan layer but lacking the outer lipopolysaccharide membrane typical of Gram-negative microorganisms [254]. As a result, their cytoplasmic membrane is more accessible to membrane-interacting molecules, including cationic and amphipathic peptides.

Animal liver tissue contains a highly diverse spectrum of intracellular, extracellular, and membrane-associated proteins [307]. Large-scale proteomic studies have identified thousands of proteins in liver tissue, highlighting the liver as a promising substrate for the generation of multifunctional bioactive peptides [13].

The peptide sequences of hydrolysates obtained from the same protein source can vary greatly depending on the type of enzyme used; different proteolytic enzymes have specific cleavage sites that determine the size, sequence, and biological activity of the resulting peptides [308]. Endopeptidases such as pepsin and papain hydrolyze peptide bonds within the protein rather than at the N- or C-termini. Pepsin, a member of the aspartic protease family, selectively cleaves bonds near aromatic amino acids, including phenylalanine (Phe), tryptophan (Trp), and tyrosine (Tyr) residues [309–311] . These aromatic amino acids have antioxidant properties, as they can chelate metal ions and neutralize lipid radicals [312, 313].

Papain is a broad-spectrum plant cysteine protease with a high degree of hydrolysis and the ability to generate low-molecular-weight peptides that often exhibit diverse biological activities [307, 308]. This enzyme typically cleaves peptide bonds near arginine (Arg) or lysine (Lys) residues, especially when followed by a hydrophobic amino acid [313]. Due to their different substrate specificity, pepsin and papain hydrolysates exhibit different peptide profiles and different biological properties. Differences in enzyme specificity may also explain the diversity of peptides found in porcine liver hydrolysates in our study. Peptides generated by pepsin were mainly derived from intracellular proteins, such as peroxiredoxin-6 (PRDX6), TPR nucleoproteins, DENND4C, EEF1A1 or AKAP4. Peptides identified in papain hydrolysates were more often associated with membrane or metabolic proteins, including CDH9, AOX1, UNC80, TENM2 or ABCA14. Such differences reflect the different substrate specificity of enzymes and lead to different peptide profiles of the hydrolysates.

Peptides with potent antioxidant activity often contain a higher proportion of hydrophobic amino acids compared to hydrophilic ones, which may enhance their ability to neutralize free radicals [314]. Recent studies have shown that hydrophobic amino acid residues, especially as tryptophan and isoleucine, significantly enhance peptide adsorption in lipid bilayers due to strong interactions with the hydrophobic core of membranes [315]. In addition to hydrophobicity, the presence of specific aromatic amino acid residues in peptide sequences is also considered an important structural factor contributing to antioxidant activity. Aromatic residues enhance antioxidant capacity because their conjugated π-electron systems allow for extensive delocalization of unpaired electrons across the aromatic ring, thereby stabilizing radical intermediates and reducing their overall reactivity [316, 317]. Several peptides identified in this study, including FDMAAVLQQVL, LPWGSRGA, KNLHNITGVL, and MPVRVVSPDN, contained a high proportion of hydrophobic amino acids such as valine, leucine, and isoleucine, which are known to enhance the radical-scavenging ability of antioxidant peptides. The presence of aromatic residues such as phenylalanine and tryptophan in peptides such as LPWGSRGA, FPFSV, and DMHTKPWVR may further contribute to antioxidant activity by stabilizing free radicals through resonance structures. The obtained results indicate that papain-induced hydrolysis promotes the generation of structurally diverse bioactive peptides enriched in hydrophobic and aromatic amino acid residues, which may contribute to the enhanced antioxidant and antimicrobial properties of liver protein hydrolysates.

4.4. Formulation and evaluation of functional gummy candies: microbiological, bioactive, physicochemical, and sensory properties

Microbiological quality is a critical parameter in evaluating the safety, stability, and shelf life of gummy candy formulations [318]. The microbiological quality of the agar-based gummy samples was assessed over a 21-day storage period. The microbiological quality of the agar-based gummy samples was assessed over a 21-day storage period. The samples exhibited excellent microbiological stability, with no aerobic mesophilic bacteria detected throughout the storage period. Previous studies on agarose hydrogels have demonstrated that bacterial growth is closely governed by the physicochemical properties of the matrix, including water-binding capacity, microstructure, and mechanical stiffness. These factors influence free-water availability and nutrient diffusion within the hydrogel network, thereby restricting bacterial proliferation [319, 320]. Mesophilic aerobic microorganisms, bacteria, yeasts, and other microscopic fungi are among the main factors responsible for the microbiological spoilage of food products and are often associated with contaminated raw materials, improper processing, or inadequate storage conditions [233]. Microbial growth is also strongly influenced by pH, as acidic environments ($pH < 4.5$) inhibit the growth of most pathogenic microorganisms [217]. This effect was evident in the present study, where agar-based gummies enriched with papain-derived porcine liver hydrolysates exhibited an acidic pH (3.65–3.70), which likely contributed to their enhanced microbiological stability. In addition, agar-based gelling agents form dense gel networks with lower free-water availability, which may further reduce the risk of moisture-related microbial spoilage [321].

The nutritional value of gummies is crucial for assessing their quality and their potential as healthier or functional confectionery alternatives. Their nutrient composition determines both the nutritional benefits of the product and its consumer acceptability [322]. Protein hydrolysates are widely recognised as functional food ingredients capable of enhancing the nutritional value of food products due to their high protein content and the presence of bioactive peptides with various biological activities [323] . The incorporation of porcine liver hydrolysates significantly increased the protein contents of the gummies compared with the control sample. Protein content increased from 0.04 g/100 g in the control sample to 4.48–4.56 g/100 g in the hydrolysate-enriched formulations, confirming that porcine liver hydrolysates represent an effective source of protein. Similar findings have been reported in previous studies, which demonstrated that the incorporation of protein-rich

ingredients and protein hydrolysates improves the nutritional value of gummy candies [324, 325].

The inclusion of porcine liver hydrolysates significantly affected the physicochemical and functional properties of the developed gummy formulas. Protein hydrolysates are recognised as important sources of bioactive peptides, which, depending on their molecular composition and peptide profile, can exhibit antioxidant, antimicrobial and other biological activities [27, 326].

The results of the present study demonstrated that the incorporation of porcine liver hydrolysates significantly improved the antioxidant properties of the gummies. Compared with the control sample, which exhibited relatively low $ABTS^{\bullet+}$ (8.52 ± 0.58 μmol/g) and $DPPH^{\bullet}$ (8.62 ± 0.16%) radical scavenging activity, the hydrolysate-enriched samples showed a markedly stronger antioxidant response. The highest antioxidant activity was observed in the GC5LPa24Ag sample, in which $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging activities reached 67.6 ± 0.98 μmol/g and 49.14 ± 1.10%, respectively. These results suggest that a longer hydrolysis duration promoted the formation of peptide fractions with enhanced antioxidant activity. Similar trends were reported by Duan et al. [151], who demonstrated that papain-hydrolysed liver exhibited higher $DPPH^{\bullet}$ radical scavenging activity than hydrolysates produced using pepsin or trypsin. Hidalgo et al. [327] also found that antioxidant activity increased with increasing hydrolysis time, while Verma et al. [290] reported the highest antioxidant activity in samples subjected to the longest hydrolysis period.

The choice of proteolytic enzymes and the hydrolysis conditions applied are recognized as decisive factors in the formation of antimicrobial peptides [328]. Differences in enzyme specificity directly affect the peptide profiles, which subsequently affect the biological activity of the resulting hydrolysates. Antimicrobial peptides primarily act by interacting with microbial cell membranes, causing structural disruption, increased membrane permeability, depolarization, dissipation of electrochemical gradients, and ultimately cell death [329].

The hydrolysates obtained during longer hydrolysis significantly enhanced antimicrobial activity. The GC5Pa6Ag and GC5Pa24Ag samples were characterized by significantly larger inhibition zones. The highest inhibition effect was determined in the GC5Pa24Ag sample, in which the inhibition zone against *L. monocytogenes* reached 30.5 ± 0.2 mm. In addition, a pronounced antibacterial effect was also determined in the GC5Pa6Ag sample, in which the inhibition zones against *E. coli* and *S. aureus* reached 29.0 ± 0.2 mm and 27.0 ± 0.1 mm. The results show that longer papain hydrolysis

promoted the formation of peptides with stronger antibacterial activity. Previous studies have shown that papain-derived liver protein hydrolysates exhibit antibacterial activity, which is associated with the cationic properties and hydrophobicity of the peptides. Such peptides can effectively interact with negatively charged cell surfaces and cytoplasmic membranes of microorganisms, causing membrane destabilisation, increased permeability, and cell lysis [330–332].

Low molecular weight peptides and free amino acids not only exhibit biological activity, but also have a significant impact on the sensory properties of food products, as peptides can impart sweet, bitter, umami, salty or sour tastes and modulate overall taste perception, depending on their sequence and structure [220]. Hydrolysate-enriched gummy candy samples exhibited overall acceptability scores comparable to those of the control sample, indicating that the selected hydrolysate concentrations were successfully incorporated into the product matrix without adversely affecting consumer perception of the product. Nevertheless, the sensory properties of protein hydrolysates vary depending on the enzyme used, the degree of hydrolysis, the molecular weight distribution of peptides, and their amino acid composition. Previous studies have shown that the bitterness of protein hydrolysates can be reduced by using modified starch and organic acids such as malic or citric acid [198, 218]. In addition, Bertelsen et al. reported that xylitol, sucrose, α-cyclodextrin, and maltodextrin act as effective taste-masking agents to reduce bitterness in soy protein hydrolysates [197].

Food colour can be a key factor in determining consumer perceptions of quality, influencing sensory acceptance [333]. Compared with the control sample, all hydrolysate-enriched gummies exhibited significantly lower lightness (L*) values and higher a*, b*, and c* values, indicating the development of a darker and more saturated colour. Liu et al. [334] reported that peptides and free amino acids generated during protein hydrolysis exhibit increased reactivity in Maillard reactions and may therefore contribute to enhanced non-enzymatic browning and darker colour formation in protein-based food systems. Furthermore, the degree of hydrolysis has been associated with colour development, as higher concentrations of low-molecular-weight peptides provide a greater number of reactive groups capable of participating in Maillard-type reactions, resulting in more pronounced colour changes. Zhang et al. [335] also indicate that hydrophobic peptides formed during hydrolysis exhibit higher reactivity in Maillard reactions, thus significantly contributing to the formation of darker color in protein systems.

The textural properties of the gummies were significantly influenced by the hydrolysis duration of the porcine liver hydrolysates. The significantly

higher hardness of the GC5LPa24Ag sample (4.63 mJ) suggests that prolonged papain hydrolysis may have altered the structural characteristics of the hydrolysates, thereby enhancing their interaction with the agar matrix and contributing to the formation of a firmer gel system. This tendency is consistent with previous studies on papain-hydrolysed soy protein isolate, which showed that enzymatic treatment can improve gel properties by promoting the formation of stronger and more cohesive protein networks [336]. It has also been suggested that low molecular weight peptides formed during hydrolysis can act as active components of network formation, thereby contributing to increased gel stiffness and mechanical resistance. Previous studies have also shown that peptides formed during enzymatic hydrolysis can participate in gel network formation through hydrophobic interactions and hydrogen bonds, thereby improving gel strength and structural stability [337].

Evaluation of the gummies' microstructure revealed that the control sample was homogeneous and had low porosity. In contrast, samples containing liver hydrolysates gelled with agar exhibited increased microporosity and phase separation. Meanwhile, the gummy formulations prepared with gelatin exhibited a more uniform microstructure with lower surface roughness and porosity, indicating stronger intermolecular interactions. Agar gels form a three-dimensional polysaccharide network generated through the aggregation of agarose chains into double-helical structures. These assemblies create a porous matrix that determines the mechanical and physicochemical properties of the gel. Agar gels have been reported to exhibit relatively large and irregularly shaped pores, while their microstructure is influenced by agar concentration, gelation conditions, and the presence of other components within the system [338]. Furthermore, increasing polymer concentration has been shown to promote the formation of a more homogeneous gel network and a more uniform pore size distribution [339].

CONCLUSIONS

1. The nature of the pathological lesions identified in animal organs during veterinary post-mortem examination did not have a significant impact on chemical or biological safety. However, these pathological lesions substantially affected the nutritional composition of animal-derived products and their suitability for protein hydrolysate production.
   1.1. The pathological lesions identified in organs of bovine and ovine were mostly associated with the digestive system, whereas in porcines, they were primarily related to the cardiovascular system, with myocardial haemorrhages and cases of endocarditis being predominant. However, the most frequently observed pathological lesions across all animal species were fibrotic, degenerative, and hyperaemic lesions in the liver, which resulted in the largest proportion of by-products being classified as Category 3 animal by-products.
   1.2. Regardless of the identified lesions, all analysed porcine, ovine, and bovine organs complied with the chemical and microbiological safety requirements established by EU legislation. In all examined samples, the concentrations of mercury (Hg), lead (Pb), cadmium (Cd), and organochlorine pesticide residues were below the maximum limits established by the European Union.
   1.3. The identified lesion in the organs of all animal species affected the nutritional composition of products classified as Category 3 animal by-products. It can be stated that fibrotic, degenerative, and hyperaemic liver lesions had a least impact on the nutritional composition of the raw material, as significantly higher contents of protein, essential amino acids, and polyunsaturated fatty acids were determined in the liver compared to other organs exhibiting pathological lesions ($p < 0.05$).
   1.4. Based on the evaluation of the biological and chemical safety, nutritional composition, and nature of the pathological lesions in the examined animal organs, porcine, ovine, and bovine livers intended for human consumption or classified as Category 3 animal by-products due to fibrotic, degenerative, or hyperaemic lesions were identified as the most suitable raw materials for protein hydrolysate production. Only hearts classified as Category 3 animal by-products with haemorrhagic lesions were considered suitable for protein hydrolysate production. Kidneys and lungs were identified as the

least suitable raw materials for enzymatic hydrolysis due to their lower protein and higher fat contents.

2. Enzymatic hydrolysis using "Flavourzyme," pepsin, and papain demonstrated that the degree of protein hydrolysis (DH) was significantly affected by enzyme type and hydrolysis duration ($p < 0.001$). In all liver samples, DH increased consistently from 3 h to 24 h, with the highest values observed after 24 h of hydrolysis. Among the enzymes tested, papain yielded the highest DH values across all analysed offal, whereas pepsin exhibited moderate hydrolysis efficiency. "Flavourzyme" demonstrated the lowest hydrolytic efficiency, resulting in the lowest DH values, and was therefore excluded from further experiments.

3. The enzymatic hydrolysis of porcine liver from edible offal (E) and Category 3 (C3) animal by-products using papain and pepsin for 24 h represents a promising method to produce biologically active peptide hydrolysates with antioxidant and antibacterial properties for the development of functional food products:
   3.1. The antioxidant and antibacterial activity of all analysed liver hydrolysates was significantly influenced by the enzyme type, hydrolysis duration, and raw material group (E and C3) ($p < 0.001$). Hydrolysis with papain resulted in significantly higher antioxidant activity in liver peptides and stronger effects against *Listeria monocytogenes* and *Bacillus cereus* than hydrolysis with pepsin.
   3.2. LC–MS/MS analysis enabled the identification of putative bioactive peptides, with the highest antioxidant potential observed in edible porcine liver hydrolysates. In these hydrolysates, the peak areas of the identified peptides showed strong positive correlations with $DPPH^{\bullet}$ radical scavenging activity ($r = 0.857–0.896$, $p < 0.01$) and $ABTS^{\bullet+}$ radical scavenging activity ($r = 0.885–0.889$, $p < 0.01$).

4. Selected porcine liver protein hydrolysates obtained after enzymatic hydrolysis with papain were successfully incorporated into the development of functional gummies. The incorporation of hydrolysates enhanced the antioxidant and antibacterial properties of the gummies and increased their protein content. The highest biological activity was observed in agar-based gummies supplemented with 5% porcine liver protein hydrolysate obtained after 24 h of enzymatic hydrolysis with papain. Despite hydrolysate-induced changes in colour and texture, the overall sensory acceptability remained unaffected. These findings demonstrate the potential of porcine liver protein hydrolysates as functional ingredients for the development of functional food products.

RECOMMENDATIONS

1. Livers intended for human consumption and classified as Category 3 animal by-products, exhibiting fibrotic, degenerative, and hyperaemic lesions and characterised by high protein content and a favourable essential amino acid profile, represent a promising raw material for the production of peptide hydrolysates. To ensure the targeted utilization of Category 3 livers for the production of functional ingredients, a more detailed differentiation based on pathological lesions and the biological value of the raw material is recommended.
2. To obtain porcine liver peptide hydrolysates exhibiting the highest antioxidant and antimicrobial activity, it is recommended to apply 24 h enzymatic hydrolysis using papain, following freeze-drying of the raw material. Peptide hydrolysates obtained in this manner may be applied in the production of functional foods or protein supplements.

SANTRAUKA

ĮVADAS

Pasaulio gyventojų skaičiui nuolat augant – 2024 m. jis siekė 8,2 mlrd., o iki 2050 m., prognozuojama, išaugs iki 9,7 mlrd. – vis svarbesni tvarūs, maistingi baltymų šaltiniai ir efektyvus esamų biologinių išteklių panaudojimas [1]. Pasauliniu mastu maisto nuostoliai ir švaistymas laikoma viena didžiausių šiuolaikinių maisto sistemų neefektyvumo problemų, turinčių reikšmingą poveikį aplinkai, ekonomikai ir mitybai [2]. Maisto ir žemės ūkio organizacijos duomenimis, kasmet pasaulyje prarandama arba iššvaistoma apie 1,3 mlrd. tonų maisto [3]. Mėsos perdirbimo pramonėje daugelis šalutinių produktų, tokių kaip subproduktai, kaulai ir kraujas, dažnai nepakankamai panaudojami arba pašalinami kaip atliekos [4]. Toks nepakankamas panaudojimas iš dalies aiškinamas tuo, kad gyvulio gyvasis svoris skirstomas į maistui tinkamas ir netinkamas dalis, o šalutiniai produktai sudaro apie 66 proc. galvijų, 52 proc. avių ir iki 80 proc. kiaulių skerdenos svorio [5]. Kepenys yra vienas iš organų, dažniausiai atmetamų atlikus veterinarinį patikrinimą po skerdimo, o jų patologiniai pažeidimai sudaro didžiausius ekonominius nuostolius (58,64 proc.) [6]. Dėl to ne tik prarandamos vertingos maisto medžiagos, bet ir kyla aplinkosaugos problemų, įskaitant didesnes šiltnamio efektą sukeliančių dujų emisijas, intensyvesnį vandens ir žemės naudojimą ir taršą, susijusią su netinkamu atliekų tvarkymu [7]. Nors šalutiniai gyvūniniai produktai, ypač kepenys ir širdis, turi daug maistinių ir bioaktyviųjų medžiagų, jie vis dar nepakankamai panaudojami, todėl tai gali būti perspektyvi žaliava gaminant funkcionaliuosius ingredientus [8]. Gyvūninės biomasės valorizacija tampa svarbiu prioritetu, nes vis dažniau pripažįstama, kad vertingų junginių išgavimas iš šalutinių mėsos produktų – veiksminga atliekų mažinimo ir maisto medžiagų panaudojimo gerinimo strategija [9, 10]. Vis dėlto, nors šių organų biocheminė sudėtis gausi, jų valorizacijos potencialas išlieka nepakankamai ištirtas.

Europos riebalų ir gyvūninių šalutinių produktų perdirbėjų asociacijos (EFPRA) duomenimis, Europos mėsos perdirbimo įmonėse kasmet susidaro apie 5 mln. tonų 1 ir 2 kategorijų gyvūninių šalutinių produktų bei apie 12 mln. tonų 3 kategorijos gyvūninių šalutinių produktų [11]. Tačiau reikšminga dalis 3 kategorijos šalutinių produktų skerdyklose klasifikuojama ir eksportuojama kaip 1 kategorijos medžiaga, taip sumažinant jų ekonominį ir maistinį potencialą [12]. Dėl to šios žaliavos dažnai nukreipiamos ne maisto reikmėms, nors jose gausu didelės biologinės vertės baltymų, turinčių reikšmingą maistinį potencialą žmogaus mityboje. Apskaičiuota, kad panaudojus bent pusę šių išteklių būtų galima papildomai gauti apie 200 mln. kilogramų

baltymų per metus [13]. 3 kategorijos skerdyklų šalutinių produktų naudojimą riboja griežti reglamentavimo ir saugos reikalavimai, mažinantys jų panaudojimo galimybes [12, 14]. Nors maistinis ir funkcinis potencialas yra didelis, 3 kategorijos skerdyklų šalutiniai produktai išlieka nepakankamai ištirti ir menkai panaudojami, kadangi dabartiniai šių antrinių mėsos žaliavų perdirbimo bei valorizacijos metodai nėra pakankamai išplėtoti ir plačiai taikomi praktikoje [4].

Šios aplinkosaugos ir visuomenės sveikatos problemos sustiprina poreikį iš naujo įvertinti šalutinių mėsos produktų panaudojimą, laikant juos ne atliekomis, o vertinga žaliava. Pasak Europos riebalų perdirbėjų ir lydytojų asociacijos, fermentinė baltymų hidrolizė leidžia iš mėsos šalutinių produktų išskirti bioaktyvias molekules ir kurti didelės pridėtinės vertės ingredientus [15, 16]. Šis procesas suteikia galimybę pagerinti jų biologinį prieinamumą ir funkcines savybes, tokias kaip tirpumas, klampumas, emulsinimas ir gelio formavimas, taip išgaunant bioaktyviuosius junginius ir kuriant didelės pridėtinės vertės ingredientus [17, 18]. Gyvūniniai šalutiniai produktai laikomi perspektyvia žaliava bioaktyvių hidrolizatų gamybai, nes juose esantys junginiai pasižymi reikšmingomis fiziologinėmis savybėmis ir atlieka svarbias biologines funkcijas žmogaus organizme [17]. Organinių šalutinių produktų biocheminė sudėtis skiriasi, priklausomai nuo gyvulių rūšies: kiaulių kepenyse ir širdyse gausu metabolinių fermentų, hemo turinčių baltymų ir bioaktyviųjų junginių, galvijų organuose daugiau struktūrinių baltymų ir jungiamojo audinio komponentų, o avių organai pasižymi savitu proteominiu ir aminorūgščių profiliu [19, 20]. Šie tarprūšiniai skirtumai daro įtaką substratų prieinamumui fermentiniam skaidymui ir lemia hidrolizės metu susidarančių peptidų profilius [21].

Dėl didelio baltymų kiekio mėsos šalutiniai produktai yra puikus substratas įvairų biologinį aktyvumą turintiems peptidams susidaryti [22, 23]. Bioaktyvūs peptidai, fermentinės hidrolizės metu susidarantys iš didesnių baltymų molekulių, gali pasižymėti antioksidaciniu, antimikrobiniu, antihipertenziniu, priešuždegiminiu ir imunomoduliaciniu poveikiu [24, 25]. Dėl šių savybių peptidai pastaraisiais metais sulaukia vis didesnio dėmesio kaip fiziologiškai aktyvios molekulės, turinčios terapinę vertę. Dėl didelio specifiškumo jie saugūs, geriau toleruojami ir yra efektyvesni, todėl jie laikomi perspektyvia medžiaga šiuolaikinėje vaistų kūrimo praktikoje [22]. Bioaktyvius peptidus gaunant iš šalutinių mėsos produktų, galima reikšmingai apriboti atliekų srautus, taip mažinant poveikį aplinkai ir didinant mėsos gamybos sistemų tvarumą [26]. Didėjant metabolinių ligų ir su žarnynu susijusių sveikatos sutrikimų paplitimui, dar labiau reikia naujų mitybos (dietos) intervencijų, galinčių moduliuoti žarnyno mikrobiotą ir gerinti visuomenės sveikatą [13, 18]. Iš šalutinių mėsos produktų gauti bioaktyvūs peptidai gali veikti žarnyno

mikrobiotos pusiausvyrą, stiprinti žarnyno barjero vientisumą ir teigiamai moduliuoti imuninę funkciją, todėl jie gali būti naudojami kaip funkciniai priedai skiriant gydomąsias dietas [27]. Be to, iš šalutinių mėsos produktų gautus bioaktyvius peptidus integruojant į funkcinius maisto produktus ar maisto papildus gali būti atvertos reikšmingos, tačiau dar neišnaudotos ekonominės galimybės, ypač regionuose, kur mėsos pramonė yra reikšminga, o atliekų tvarkymo sistemos nepakankamai išvystytos [15]. Nors mokslinis susidomėjimas auga, dabartinės žinios apie peptidų išgavimo efektyvumą, fermentinės hidrolizės sąlygų optimizavimą ir gautų hidrolizatų funkcinį integravimą į maisto sistemas išlieka fragmentiškos ir nepakankamai išplėtotos [16].

Valgomųjų subproduktų ir 3 kategorijos gyvūninių šalutinių produktų kokybė, sauga ir funkcionalumas išlieka esminiai veiksniai, siekiant juos efektyviai panaudoti maisto sistemose. Standartizuotų metodų stoka vertinant 3 kategorijos pažeidimus po skerdimo gali riboti tikslų šių žaliavų klasifikavimą ir apsunkinti veiksmingą jų atranką tolesniam naudojimui bei perdirbimui. Todėl, siekiant užtikrinti jų saugų ir efektyvų panaudojimą, būtinas išsamus patologinių pokyčių, biologinės, cheminės ir mikrobiologinės saugos bei maistinės sudėties įvertinimas. Didėjantis dėmesys tvariam išteklių naudojimui ir atliekų mažinimui atskleidžia subproduktų ir 3 kategorijos gyvūninių šalutinių produktų potencialą, siekiant išgauti bioaktyviuosius junginius ir kurti didesnės pridėtinės vertės produktus. Taikant fermentinę hidrolizę, galima išgauti pakankamai stabilius peptidus, pasižyminčius potencialiu antioksidaciniu ir antibakteriniu aktyvumu. Šiuos peptidinius hidrolizatus galima pritaikyti kuriant funkcionaliuosius ingredientus ir inovatyvius maisto produktus, taip užtikrinant tvaresnį gyvūninių žaliavų perdirbimą mėsos pramonėje.

Darbo tikslas – įvertinti gyvūninių subproduktų saugą ir jų tinkamumą perdirbimui į baltymų hidrolizatus bei funkcionaliojo maisto produkto kūrimui.

Darbo uždaviniai

1. Įvertinti po skerdimo veterinarinio tyrimo metu nustatytus gyvūninių subproduktų (kiaulių, galvijų ir avių) patologinius pokyčius ir jų poveikį biologinei bei cheminei saugai, maistinei sudėčiai, žaliavos tinkamumui toliau perdirbti.
2. Pritaikyti optimalų fermentinės hidrolizės modelį atrinktų gyvūninių subproduktų žaliavai perdirbti, išgaunant funkcionaliosiomis savybėmis pasižyminčius baltyminius hidrolizatus.

3. Įvertinti gyvūninių subproduktų hidrolizatų antioksidacines, antibakterines savybes, peptidų profilius ir jų pritaikymo galimybes kuriant funkcionaliuosius maisto produktus.
4. Sukurti naujo produkto prototipą, įvertinti jo saugą, maistinę sudėtį ir funkcionaliąsias savybes, priimti moksliniais tyrimais pagrįstus sprendimus dėl gyvūninių subproduktų panaudojimo kuriant funkcionaliuosius maisto produktus.

Mokslinis naujumas

Šiame tyrime nagrinėjamos nepakankamai panaudojamos žaliavos – 3 kategorijos šalutiniai gyvūniniai produktai ir valgomieji subproduktai, kurie pasižymi didele biologine verte – yra aukštos kokybės baltymų ir bioaktyviųjų junginių šaltinis. Pirmą kartą atliktas išsamus šių žaliavų palyginamasis vertinimas, akcentuojant jų saugą, maistinę vertę ir technologinį pritaikomumą maisto sistemose.

Pasirinktos žaliavos analizuotos svarbiausiais biologinės, cheminės, mikrobiologinės saugos ir patologinių pokyčių aspektais, taip nustatant jų tinkamumą toliau perdirbti. Taikant fermentinę hidrolizę gauti bioaktyvūs peptidiniai hidrolizatai, pasižymintys antioksidacinėmis, antibakterininėmis ir funkcionaliosiomis savybėmis, detaliai įvertinus fermento tipo ir hidrolizės laipsnio įtaką hidrolizatų biologiniam aktyvumui. Peptidų sekų analizė leido identifikuoti potencialiu biologiniu aktyvumu pasižyminčius peptidus ir atskleisti jų pritaikomumą kuriant funkcionaliuosius maisto produktus.

Sukurti gyvūninių subproduktų peptidiniai hidrolizatai pirmą kartą inkorporuoti kaip funkcionalieji priedai į kramtomųjų guminukų matricas, sukūrus inovatyvių maisto produktų prototipus.

Šio darbo rezultatai suteikia naujų mokslinių žinių apie 3 kategorijos šalutinių gyvūninių produktų ir valgomųjų subproduktų pritaikymo galimybes, parodo jų svarbą iš tvarių ir ekonomiškai vertingų žaliavų kuriant funkcionaliuosius maisto ingredientus bei produktus.

2. TYRIMO METODAI IR MEDŽIAGOS

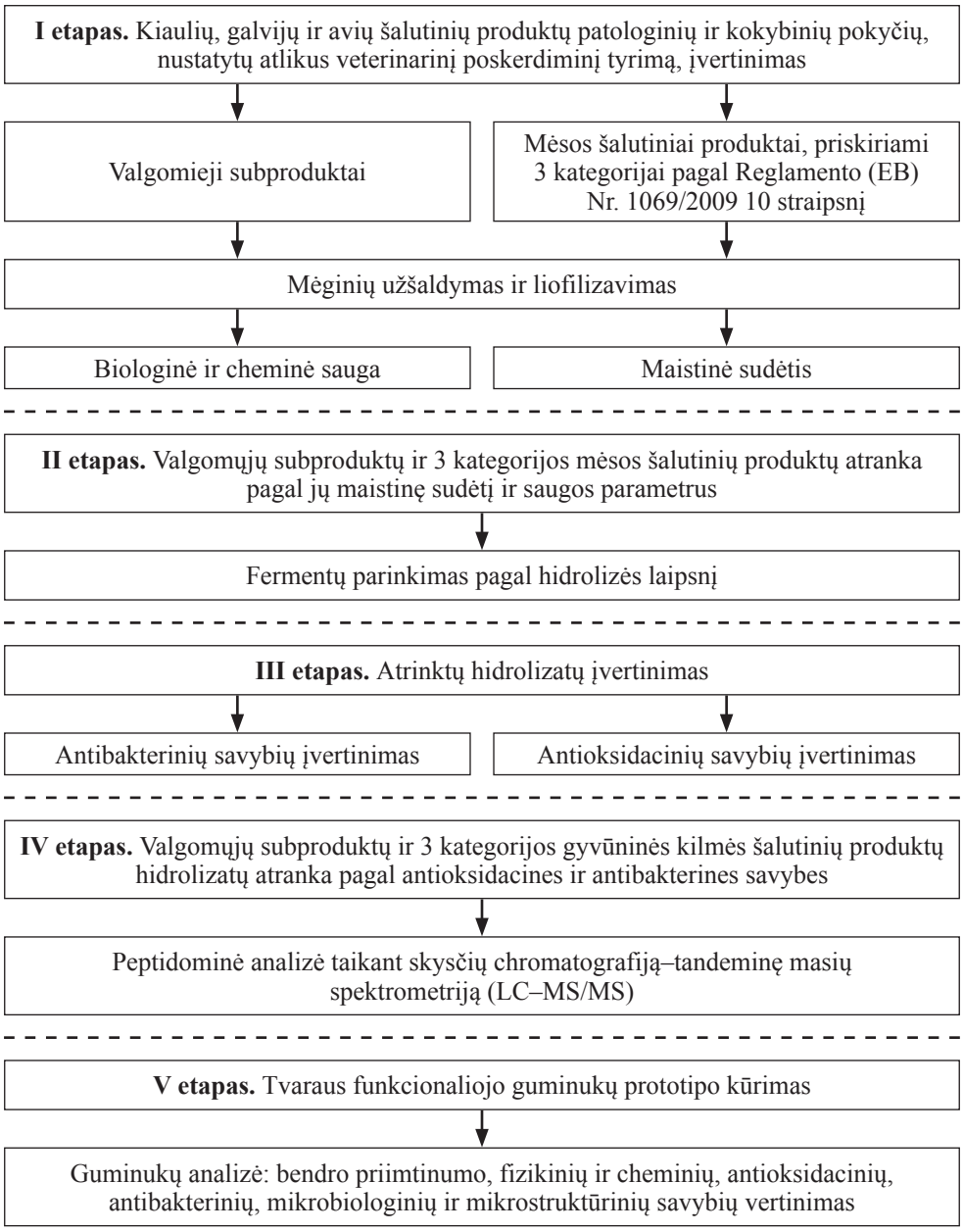
Tyrimas atliktas Maisto saugos ir kokybės katedroje, Veterinarijos fakultete, Lietuvos sveikatos mokslų universitete 2021–2025 m. Visi tyrimui reikalingi mėginiai surinkti dviejose Lietuvos skerdyklose.

2.1. Etikos pareiškimas

Šiam tyrimui nereikėjo leidimo ar etinio patvirtinimo pagal Europos Parlamento ir Tarybos direktyvą 2010/63/ES, nes gyvi gyvūnai eksperimentiniais tikslais nenaudoti ir tyrimo tikslais nebuvo specialiai nužudyti. Visi mėginiai (kepenų, širdžių, plaučių, inkstų) iš ūkinių gyvūnų, paskerstų komercinės maisto gamybos metu, surinkti jiems mirus. Gyvūnai buvo paskersti laikantis standartinės pramonės praktikos, prižiūrint Valstybinei maisto ir veterinarijos tarnybai. Visos su gyvūnais susijusios procedūros buvo atliekamos laikantis 2009 m. rugsėjo 24 d. Europos Sąjungos reglamento (EB) Nr. 1099/2009 dėl žudomų gyvūnų apsaugos (Europos Sąjungos oficialusis leidinys L 303/1) reikalavimų. Tyrime naudoti šalutiniai gyvūniniai produktai buvo tvarkomi laikantis Europos Parlamento ir Tarybos reglamento (EB) Nr. 1069/2009, kuriuo apibrėžiamos nuostatos dėl šalutinių gyvūninių produktų ir jų gaminių tvarkymo ir sveikatos apsaugos. Surinkti biologiniai mėginiai tvarkyti laikantis galiojančių biologinės saugos ir laboratorinių tyrimų standartų. Mėginių rinkimas, transportavimas ir saugojimas atliktas užtikrinant jų tinkamumą analizei ir saugant nuo galimo užteršimo.

2.2. Tyrimo planas

Šioje disertacijoje daugiausia dėmesio skirta šalutinių gyvūninių produktų saugos ir kokybės vertinimui bei jų pritaikymui funkcionaliųjų maisto produktų gamyboje. Bendras tyrimo planas ir eksperimentinių procedūrų seka pateikti 2.2.1 pav. Tyrimas atliktas keliais etapais, įskaitant valgomųjų subproduktų ir šalutinių gyvūninių produktų (kiaulių, galvijų ir avių) saugos, maistinės sudėties ir tinkamumo perdirbti vertinimą; pasirinktų žaliavų fermentinę hidrolizę ir peptidinių hidrolizatų gavybą, gautų hidrolizatų antioksidacinių, antibakterinių ir funkcionaliųjų savybių vertinimą bei funkcionaliųjų maisto produktų prototipų kūrimą ir vertinimą.



2.2.1 pav. *Tyrimo schema*

2.3. Veterinarinis tyrimas po skerdimo

Organų patologiniai pokyčiai vertinti atliekant veterinarinį tyrimą po skerdimo X ir Y skerdyklose. Iš viso ištirta 600 kiaulių, galvijų ir avių organų mėginių (kepenų, širdžių, inkstų ir plaučių); avių mėginiai buvo surinkti skerdykloje Y. Galvijų grupėje (n = 600) karvės sudarė 54,5 proc. (n = 327), buliai – 27,0 proc. (n = 162), o telyčios – 18,5 proc. (n = 111).

Veterinarinis tyrimas po skerdimo atliktas vadovaujantis Komisijos įgyvendinimo reglamento (ES) 2019/627 nuostatomis [237]. Skerdenų ir subproduktų tinkamumas maistui vertintas taikant standartizuotus tyrimo metodus – vizualinį vertinimą, palpaciją ir, esant poreikiui, audinių pjūvius. Sprendimai dėl subproduktų panaudojimo priimti vadovaujantis kokybės sistemos darbo instrukcija KT–2–3–1–D1 „Mėsos ir kitų skerdimo produktų tinkamumo maistui vertinimas ūkinių gyvūnų skerdyklose“, patvirtinta Valstybinės maisto ir veterinarijos tarnybos direktoriaus 2020 m. balandžio 22 d. įsakymu Nr. B1-270 [238].

Subproduktų priskyrimas atitinkamoms kategorijoms buvo atliekamas vadovaujantis Reglamento (EB) Nr. 1069/2009 reikalavimais [239].

Nustatyti šalutinių gyvūninių produktų pokyčiai buvo registruojami ir klasifikuojami, atsižvelgiant į jų pobūdį bei priskiriant juos atitinkamoms šalutinių gyvūninių produktų kategorijoms (2.3.1 lentelė).

2.3.1 lentelė. *Patologinių pokyčių klasifikacija pagal gyvūninių šalutinių produktų kategorijas*

Organas	**2 kategorijos šalutiniai gyvūniniai produktai**	**3 kategorijos šalutiniai gyvūniniai produktai**
Kepenys	Kepenų abscesai; kepenų fasciolіozė; lėtinis cholangitas; židininė kepenų nekrozė	Riebalinė kepenų degeneracija; kepenų hiperemija; kepenų fibrozė
Širdys	Fibrininis perikarditas; endokarditas	Miokardo hemoragijos
Plaučiai	Pneumonija; aspiracinė pneumonija	Plaučių emfizema
Inkstai	Lėtinis intersticinis nefritas; nefritas	Inkstų cistos

2.3.1. Valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų mėginių paruošimas

Mėsos šalutiniai produktai, pasižymintys patologiniais pokyčiais, surinkti ir klasifikuoti kaip 3 kategorijos šalutiniai gyvūniniai produktai, laikantis Reglamento (EB) Nr. 1069/2009 reikalavimų. Į šią grupę buvo įtraukti kepenų mėginiai (n = 8 × 3), kuriuose nustatyta riebalinė kepenų degeneracija, hiperemija ir fibrozė; širdies mėginiai (n = 8 × 3), kuriuose nustatytos miokardo hemoragijos; plaučių mėginiai (n = 8 × 3), kuriuose nustatyta plaučių emfizema; bei inkstų mėginiai (n = 8 × 3), kuriuose nustatytos inkstų cistos.

Mėginiai, kuriuose tyrimo po skerdimo metu nenustatyta patologinių pokyčių, buvo atrinkti ir priskirti valgomiesiems subproduktams (tinkamiems žmonių maistui). Kontrolinę grupę sudarė kiaulių, avių ir galvijų kepenų, inkstų, širdžių ir plaučių mėginiai (n = 8 × 3). Mėginiai į laboratoriją buvo transportuojami per 24 val. po skerdimo ir laikomi sandariuose induose 4 °C temperatūroje iki tolesnių tyrimų. Prieš liofilizavimo procedūrą 3 kategorijos mėsos šalutiniai produktai ir valgomieji šalutiniai gyvūniniai produktai buvo supjaustyti 2 × 2 cm dydžio gabalėliais.

2.3.2. Liofilizavimas

Valgomieji subproduktai ir 3 kategorijos šalutiniai gyvūniniai produktai, apibrėžti Reglamento (EB) Nr. 1069/2009 10 straipsnio 4 dalyje, buvo greitai užšaldyti −35 °C temperatūroje 3 val. naudojant „Liebherr“ šaldiklį („LGv 5010 MediLine“), o vėliau liofilizuoti liofilizatoriuje („Harvest Right“, North Salt Lakas, Juta, JAV) −80 °C temperatūroje esant 20 Pa vakuumo slėgiui. Praėjus 72 val. po liofilizavimo, mėginiai buvo sumalti laboratoriniu malūnu („Fritsch Pulverisette 14“, Idaras-Obersteinas, Vokietija), veikiančiu 6000 aps./min. greičiu, ir persijoti per 200 μm akučių sietą.

2.4. Valgomųjų subproduktų ir 3 kategorijos produktų maistinės sudėties nustatymas

2.4.1. Baltymų ir riebalų kiekio nustatymas

Baltymų kiekis nustatytas Kjeldahlio metodu pagal standartą LST ISO 937:2023 „Mėsa ir mėsos produktai. Azoto kiekio nustatymas“ [240]. Riebalų kiekis analizuotas taikant standartinį LST ISO 1443:2000 metodą, skirtą bendram riebalų kiekiui mėsoje ir mėsos produktuose nustatyti [241]. Drėgmės kiekis nustatytas pagal standartą LST ISO 1442:2023 „Mėsa ir mėsos produktai. Drėgmės kiekio nustatymas (etaloninis metodas)“ [242].

2.4.2. Riebalų rūgščių sudėties nustatymas

Riebalų rūgščių sudėtis nustatyta naudojant standartizuotas analitines procedūras. Mėginiai paruošti pagal LST EN ISO 12966-2:2017 standartą „2 dalis. Riebalų rūgščių metilo esterių paruošimas“ [243], o chromatografinė analizė atlikta vadovaujantis ISO 12966-1:2014 standartu [244]. Riebalų rūgščių metilo esteriai (FAME) buvo analizuojami naudojant dujų chromatografą „PerkinElmer Clarus 680“, sujungtą su masių spektrometru „PerkinElmer Clarus SQ8T“ („PerkinElmer, Inc.“, Norvolkas, Konektikutas, JAV). Analizei naudota chromatografinė kolonėlė SP-2560 (100 m × 0,25 mm × 0,2 μm; „Sigma-Aldrich“).

Chromatografinės kolonėlės temperatūros programa buvo tokia: pradinė temperatūra – 60 °C (išlaikant 1 min.), vėliau temperatūra didinta 12 °C/min. greičiu iki 250 °C ir palaikoma 10 min. Masių spektrometro temperatūros programa buvo 5 °C/min. iki 300 °C, temperatūrą palaikant 20 min. Injektoriaus temperatūra buvo nustatyta 250 °C. Į injektorių buvo suleidžiama 1 µl paruošto mėginio iš viršutinės atsiskyrusios metilo esterių fazės.

Kiekybinis nustatymas atliktas naudojant kalibracinę kreivę, sudarytą su „Supelco 37 Component FAME Mix“ standartu („Merck & Co.“, Darmštatas, Vokietija). Nustatytos riebalų rūgštys buvo suskirstytos į sočiąsias (SFA), mononesočiąsias (MUFA) ir polinesočiąsias (PUFA). PUFA papildomai suskirstytos į omega-6 ir omega-3 pogrupius pagal pirmojo dvigubo ryšio padėtį. Nesočiųjų riebalų rūgščių (UFA) kiekis apskaičiuotas sudedant MUFA ir PUFA kiekius.

2.4.3. Lipidų indeksų apskaičiavimas

Lipidų sveikatingumo indeksai buvo vertinami apskaičiuojant sočiųjų riebalų rūgščių (SFA), mononesočiųjų riebalų rūgščių (MUFA) ir polinesočiųjų riebalų rūgščių (PUFA) sumas bei nustatant nepakeičiamąsias riebalų rūgštis (EFA) [245]. Aterogeninis indeksas (AI) ir trombogeninis indeksas (TI) apskaičiuoti pagal Ulbrichto ir Southgate'o metodiką [246]. Hipocholesteroleminis ir hipercholesteroleminis santykis (h/H) nustatytas pagal Fernandezo ir kt. metodą [247].

$$AI = \frac{C12{:}0 + 4 \cdot C14{:}0 + C16{:}0}{\Sigma MUFA + \Sigma PUFA}$$

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0{,}5 \cdot \Sigma MUFA + 0{,}5 \cdot \Sigma(n-6PUFA) + 3 \cdot \Sigma(n-3PUFA) + \left(\frac{\Sigma(n-3PUFA)}{\Sigma(n-6PUFA)}\right)}$$

$$h/H = \frac{C18{:}1 + \Sigma PUFA}{14{:}0 + 16{:}0}$$

2.4.4. Aminorūgščių sudėties nustatymas

Nuriebalintų mėginių aminorūgščių hidrolizė atlikta vadovaujantis Komisijos reglamentu Nr. 152/2009 [248]. Po hidrolizės mėginiai buvo atvėsinti iki kambario temperatūros ir toliau apdorojami. Tuomet 1 ml filtruoto hidrolizato perpilta į 100 ml matavimo kolbą kartu su 50 ml 100 mmol/ml L-2-aminosviesto rūgšties, naudotos kaip vidinis standartas. Kolbos turinys buvo praskiestas iki žymės ultragrynu vandeniu, gerai sumaišytas ir perfiltruotas naudojant 0,22 µm švirkštinį filtrą.

Derivatizacijos reagentai, kalibraciniai standartai ir mėginių paruošimas buvo atliekamas pagal „Waters AccQ Tag“ chemijos rinkinio instrukcijų vadovą [249]. Aminorūgščių analizė atlikta naudojant „Shimadzu“ žemo slėgio gradiento HPLC sistemą („Shimadzu Corp.“, Kyōto, Japonija), kurią sudarė LC-10ATVP tirpiklio tiekimo modulis, SIL-10ADVP automatinis mėginių įvediklis, CTO-10ACVP kolonėlės termostatas, RF-10AXL spektrofluorometrinis detektorius, SCL-10AVP sistemos valdiklis ir DGU-14A internetinis degazatorius. Sistemos valdymas ir duomenų surinkimas buvo atliktas naudojant „LabSolution LC“ programinę įrangą („Shimadzu Corp.“, Kiotas, Japonija).

Aminorūgščių dariniai buvo atskirti naudojant „Nova-Pak C18“ chromatografinę kolonėlę (150 × 3,9 mm, 4 μm; „Waters Corp.“, Milfordas, Masačusetsas, JAV), palaikant 37 °C temperatūrą, o injekcijos tūris siekė 10 μl. Detekcija atlikta fluorescenciniu būdu, naudojant 250 nm sužadinimo ir 395 nm emisijos bangos ilgius. Gradiento eliuavimas vykdytas 1,0 ml/min. srauto greičiu naudojant eliuentą A (100 ml „Waters AccQ Tag“ eliuento A koncentrato, praskiesto 1 l ultragryno vandens), eliuentą B (acetonitrilą) ir eliuentą C (ultragryną vandenį).

Siekiant efektyviai atskirti aminorūgščių molekules, taikytas toks gradiento algoritmas: per 0,0–0,5 min. eliuento A kiekis sumažintas nuo 100 proc. iki 98 proc., o eliuento B padidintas nuo 0 proc. iki 2 proc.; per 0,5–18,0 min. A sumažintas nuo 98 proc. iki 94 proc., o B padidintas nuo 2 proc. iki 6 proc.; per 18,0–19,0 min. A sumažintas nuo 94 proc. iki 90 proc., o B padidintas nuo 6 proc. iki 10 proc.; per 19,0–29,5 min. A sumažintas nuo 90 proc. iki 83 proc., o B padidintas nuo 10 proc. iki 17 proc. Vėliau per 29,5–35,0 min. A sumažintas nuo 83 proc. iki 0 proc., B padidintas nuo 17 proc. iki 60 proc., o C – nuo 0 proc. iki 40 proc. Mobiliosios fazės sudėtis 5,0 min. buvo palaikoma 60 proc. B ir 40 proc. C eliuentų santykiu, po to per 1,0 min. A eliuento koncentracija linijiškai padidinta nuo 0 proc. iki 100 proc., o vėliau 10,0 min. palaikyta 100 proc. A koncentracija.

Specifinės aminorūgštys identifikuotos ir kiekybiškai įvertintos lyginant atskirtų mėginio komponentų sulaikymo laikus bei smailių plotus su standartinio tirpalo duomenimis.

2.5. Valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų maistinės sudėties vertinimas

2.5.1. Mikrobiologinė analizė

Bendras aerobinių mikroorganizmų skaičius buvo nustatytas „Plate Count Agar“ terpėje (PCA, „Liofilchem“, Italija), mėginius inkubuojant 30 °C temperatūroje 72 val. laikantis EN ISO 4833-1:2013 standarto [250]. *Enterobacteriaceae* šeimos bakterijos buvo skaičiuojamos „Violet Red Bile Glucose Agar“ terpėje (VRBG, „Liofilchem“, Italija), inkubuojant 37 °C temperatūroje 24 val., o rezultatai patvirtinti gliukozės fermentacijos ir oksidazės aktyvumo testais pagal EN ISO 21528-2:2017 standartą [251].

Escherichia coli nustatymas atliktas „Tryptone Bile X-glucuronide Agar“ terpėje (TBX, „Biolife“, Monca, Italija), inkubuojant 44 °C temperatūroje 24 val. pagal ISO 16649-2:2001 standartą [252]. *Listeria monocytogenes* nustatymas (25 g mėginio) atliktas „Listeria agar“ terpėje pagal Ottaviani'io ir Agosti'io metodą (ALOA, „Liofilchem“, Italija) laikantis EN ISO 11290-1:2017 standarto [253].

Salmonella spp. nustatymas (25 g mėginio) buvo atliktas pagal ISO 6579-1:2017 standartą, naudojant selektyvų pasėjimą „Xylose Lysine Deoxycholate Agar“ terpėje (XLD, „Liofilchem“, Italija) ir „Brilliant Green Agar“ terpėje (BGA, „EO Labs“, Škotija, Jungtinė Karalystė).

2.5.2. Pesticidų likučių nustatymas

Kiekvienas mėginys buvo sumaltas iki smulkių miltelių, o 5,0 ± 0,1 g pasverta į 50 ml polipropileninį centrifuginį mėgintuvėlį. Tada į mėginį įpilta 220 µl vidinio standarto (TPP) tirpalo ir 7,8 ml acetonitrilo, bendrą tūrį padidinus iki 10 ml. Įdėjus druskų mišinį, mėginys intensyviai kratytas 1 min., po to centrifuguotas 5 min. esant ≥ 3000 g.

Viršutinė acetonitrilo fazė (apie 7 ml) buvo perkelta į 12 ml PP mėgintuvėlį ir laikyta šaldiklyje (≤ –18 °C) mažiausiai 2 val. Vėliau 5 ml atšaldytą ekstraktą perkėlus į naują 15 ml PP mėgintuvėlį, kuriame buvo 120 mg PSA ir 850 mg magnio sulfato, buvo intensyviai kratoma 45 s ir centrifuguojama 5 min. esant ≥ 3000 g.

Gauta acetonitrilo fazė perkelta į tamsaus stiklo chromatografinį buteliuką analizei atlikti.

Analizė atlikta naudojant LC–MS/MS (ESI–MS/MS) sistemą su „Waters ATLANTIS dC18“ kolonėle (2,1 × 100 mm, 3 µm). Kolonėlės temperatūra palaikyta 40 °C, tėkmės greitis – 0,25 ml/min., o injekcijos tūris – 10 µl. Bendra chromatografinės analizės trukmė buvo 30 min., taikant gradiento eliuavimo režimą: 0 min. – 100 proc. 7.5.2 fazės ir 0 proc. 7.5.1 fazės; 15 min. –

0 proc. 7.5.2 fazės ir 100 proc. 7.5.1 fazės; ši sudėtis palaikyta iki 25 min.; 26 min. – 100 proc. 7.5.2 fazės ir 0 proc. 7.5.1 fazės; 30 min. – 100 proc. 7.5.2 fazės ir 0 proc. 7.5.1 fazės.

Analitiniai duomenys apdoroti naudojant „MassLynx“ programinės įrangos paketą. Pesticidų koncentracija mėginyje apskaičiuota pagal šią formulę:

$$C = \frac{C_T \cdot V_{ACN}}{m_{mėginys}}$$

kur: C – pesticido koncentracija mėginyje (mg/kg); C_T – analitės koncentracija ekstrakte (µg/ml); V_{ACN} – acetonitrilo tūris (ml), $m_{mėginys}$ – mėginio masė (g).

2.5.3. Sunkiųjų metalų kiekio analizė

Liofilizuoti šalutinių gyvūninių produktų mėginiai buvo sumalti laboratoriniu malūnu iki homogeninės masės (dalelių dydis ≤ 0,5 mm). Malant stengtasi išvengti mėginių perkaitimo ir galimos taršos. Paruošti mėginiai iki analizės buvo laikomi sandariuose induose. Iš kiekvieno homogenizuoto mėginio į rūgštims atsparius mineralizacijos indus analitinėmis svarstyklėmis buvo pasverta po 0,50 ± 0,01 g mėginio. Sunkiųjų metalų (Hg, Cd ir Pb) analizei mėginiai buvo mineralizuojami pridedant 5 ml koncentruotos azoto rūgšties (HNO_3, 65–70 proc.) ir 1 ml vandenilio peroksido (H_2O_2, 30 proc.). Mineralizacija atlikta slėginėmis sąlygomis, temperatūrą palaipsniui didinant iki 180–210 °C ir išlaikant ją tol, kol organinė matrica visiškai susiskaidė. Po mineralizacijos mėginiai buvo atvėsinti ir perfiltruoti. Gauti tirpalai perkelti į matavimo kolbas ir praskiesti dejonizuotu vandeniu iki galutinio 25 ml tūrio. Elementinė analizė atlikta naudojant induktyviai susietos plazmos masių spektrometrą ELAN DRC II ICP-MS („PerkinElmer“ / „SCIEX“, JAV).

2.6. Valgomųjų subproduktų ir 3 kategorijos gyvūninių šalutinių produktų hidrolizatų paruošimas

Liofilizuotos kiaulių, avių ir galvijų kepenys buvo sumaltos laboratoriniu malūnu „Fritsch Grind Pulverisette 14“ iki smulkių miltelių, kurie vėliau persijoti per 200 µm sietą. Fermentinė hidrolizė atlikta naudojant papainą (≥ 30 000 USP-U/mg), pepsiną (≥ 250 U/mg) ir „Flavourzyme“® (≥ 500 LAPU/g) („Sigma-Aldrich“, Sent Luisas, Minesota, JAV). Mėginių paruošimui liofilizuoti mėginiai buvo sumaišyti su ledu santykiu 1:1.

Optimalios sąlygos kiekvienam fermentui buvo nustatytos taip: papainui – 37 °C temperatūra ir pH 6; pepsinui – 37 °C temperatūra ir pH 2,5; „Flavourzyme“® – 50 °C temperatūra ir pH 5,5. Hidrolizatų pH reguliuotas naudojant 1 N NaOH arba 1 N HCl tirpalus. Visų inkubacijos periodų metu

naudotas fermento ir substrato santykis buvo 10 g/kg (m/m). Inkubacijos trukmė – 3, 6 ir 24 val.

Po inkubacijos fermentų inaktyvacijai hidrolizatai buvo kaitinami iki 95 °C temperatūros, o vėliau atvėsinami ledo vonelėje. Hidrolizatai buvo filtruojami per filtrinį popierių ir centrifuguojami 4000 aps./min. greičiu 10 min. 4 °C temperatūroje. Kiekvieno hidrolizato supernatantas buvo užšaldytas −80 °C temperatūroje iki tolesnės analizės.

2.7. Hidrolizės laipsnio nustatymas po fermentinio apdorojimo

Kiaulių, avių ir galvijų kepenų hidrolizatai buvo vertinami nustatant TCA tirpių peptidų dalį. Hidrolizės laipsnis (DH) apskaičiuotas kaip 10 proc. (m/v) trichloracto rūgštyje (TCA) tirpių junginių procentinė dalis nuo bendro mėginio baltymų kiekio. Baltymų nusodinimas buvo atliekamas sumaišant 500 µl hidrolizato su tokiu pačiu tūriu 20 proc. (m/v) TCA tirpalo, galutinę TCA koncentraciją sumažinant iki 10 proc. (m/v) [254]. Mišinys buvo inkubuojamas 30 min. kambario temperatūroje ir vėliau centrifuguojamas 3500 aps./min. greičiu 15 min. Supernatantas buvo atitinkamai praskiedžiamas, o TCA tirpios frakcijos baltymų kiekis nustatytas pagal Hartree metodą [255]. Rezultatai išreikšti baltymų miligramais, kaip standartą naudojant galvijų serumo albuminą.

$$\text{DH (proc.)} = \left[\frac{\text{Ištirpusių baltymų kiekis tirpale 10 proc. TCA (mg)}}{\text{Bendras baltymų kiekis (mg)}}\right] \cdot 100$$

2.8. Hidrolizatų antioksidacinio aktyvumo nustatymas

2.8.1. DPPH• laisvųjų radikalų surišimo tyrimas

Hidrolizatų gebėjimas surišti DPPH• radikalus buvo vertinamas pagal Brand-Williamso ir kt. aprašytą metodą [256], taikant nedidelę modifikaciją, susijusią su naudojamo antioksidanto tirpalo kiekiu [257].

0,04 ml mėginio buvo sumaišyta su 0,96 ml 6 µM DPPH• tirpalo 75 proc. metanolyje. Absorbancija spektrofotometru „J.P. ELECTA S.A. V-1100D“ (Barselona, Ispanija) matuota praėjus trims minutėms nuo reakcijos pradžios, esant 515 nm bangos ilgiui. Kaip kontrolinis tirpalas naudotas 75 proc. metanolio tirpalas distiliuotame vandenyje.

Radikalų surišimo aktyvumas buvo apskaičiuotas pagal šią formulę:

$$\text{Antioksidacinis aktyvumas (proc.)} = \left[1 - \left(\frac{A_{\text{mėginys}}}{A_{\text{kontrolė}}}\right)\right] \cdot 100$$

2.8.2. $ABTS^{\bullet+}$ radikalo katijono surišimo tyrimas

$ABTS^{\bullet+}$ radikalo katijonas buvo paruoštas pagal [258] aprašytą metodą. Jis gautas sumaišius 7 mM vandeninį $ABTS^{\bullet+}$ tirpalą absoliučiajame etanolyje su 2,45 mM kalio persulfatu (m/m). Mišinys buvo laikomas tamsoje kambario temperatūroje 12–16 val.

Prieš naudojimą $ABTS^{\bullet+}$ tirpalas praskiestas absoliučiuoju etanoliu iki 0,8 ± 0,03 absorbancijos, kai matuojama esant 734 nm bangos ilgiui. Kontrolei naudotas reagentų blankas.

Tuomet 20 µl skirtingos koncentracijos mėsos šalutinių produktų hidrolizato buvo sumaišyta su 980 µl ABTS tirpalo ir palikta reaguoti 6 min. Gautas mišinys analizuotas naudojant spektrofotometrą „J.P. SELECTA S.A. V-1100D“ (Barselona, Ispanija).

Radikalų surišimo aktyvumas apskaičiuotas pagal šią formulę:

$$\text{Antioksidacinis aktyvumas (proc.)} = \left[1 - \left(\frac{A_{\text{mėginys}}}{A_{\text{kontrolė}}}\right)\right] \cdot 100$$

2.9. Hidrolizatų antibakterinis aktyvumas taikant agaro šulinėlių difuzijos metodą

Kiaulių, galvijų ir avių mėsos šalutinių produktų hidrolizatų antibakterinis aktyvumas buvo vertinamas taikant agaro šulinėlių difuzijos metodą. Tyrime naudotos etaloninės bakterijų padermės: *Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 ir *Escherichia coli* ATCC 25922.

Padermės buvo iš anksto kultivuojamos „Plate Count Agar“ nuolydinėse terpėse (PCA, „Liofilchem“, Roseto degli Abruzzi, Teramo, Italija) 37 °C arba 30 °C temperatūroje per naktį. Subrendusios kultūros buvo suspenduotos steriliame fiziologiniame tirpale (0,85 proc. NaCl distiliuotame vandenyje), o ląstelių tankis sureguliuotas pagal 0,5 McFarlando standartą.

Vienas mililitras standartizuotos bakterijų suspensijos buvo įmaišomas į 100 ml ištirpintos PCA terpės, atvėsintos iki 45 °C, gerai homogenizuojamas ir išpilstomas į Petri lėkšteles (90 mm, po 12 ml kiekvienoje). Terpei sustingus, agare buvo suformuojami 8 mm skersmens šulinėliai, į kuriuos įpilama po 50 µl tiriamojo tirpalo.

Lėkštelės buvo inkubuojamos 24 val. 37 °C arba 30 °C temperatūroje. Antibakterinis aktyvumas nustatytas matuojant augimo slopinimo zonų skersmenį slankmačiu, kurio tikslumas – 0,5 mm. Kaip neigiama kontrolė naudotas distiliuotas vanduo.

2.10. Minimalios slopinamosios koncentracijos (MIC) nustatymas

Liofilizuotų ir hidrolizuotų mėsos šalutinių produktų, kartu naudojant papainą, minimali slopinamoji koncentracija (MIC) nustatyta sultinio mikropraskiedimo metodu. Tiriamieji mėginiai buvo įmaišomi į „Tryptic Soy Broth“ terpę („Liofilchem Diagnostics“, Rozeto delji Abrucis, Italija), siekiant gauti pradinę 10 µg/ml koncentraciją. Vėliau buvo atliekami dvigubi nuoseklūs praskiedimai, gaunant galutines 5,0; 2,5; 1,25; 0,0625; 0,0313; 0,0156; 0,0078 ir 0,0039 µg/ml koncentracijas.

Į kiekvieną praskiedimą buvo įterpiama po 50 µl *Listeria monocytogenes* kultūros suspensijos (~$1{,}5 \times 10^8$ KSV/ml). Kontrolinius mėginius sudarė bakterijų suspensijos „Tryptic Soy Broth“ terpėje be hidrolizatų. Visi mėgintuvėliai buvo inkubuojami 37 ± 1 °C temperatūroje 24 val.

MIC buvo apibrėžta kaip mažiausia tiriamojo mėginio koncentracija, visiškai slopinusi matomą bakterijų augimą vizualinio vertinimo metu, ir išreikšta µg/ml. Tyrimai atlikti trimis pakartojimais. Dėl nereikšmingų augimo slopinimo zonų *Salmonella enterica* subsp. *enterica* serovar Typhimurium atžvilgiu, hidrolizatų (po 24 val. hidrolizės papainu) MIC nebuvo nustatyta.

2.11. Hidrolizuotų kiaulių kepenų peptidominė analizė taikant LC–MS/MS

3 µl peptidų mišinio buvo suleista į atvirkštinės fazės gaudymo kolonėlę (PST C18, 5 µm dalelių dydis, 100 Å porų dydis, 180 µm × 20 mm; „Waters Corporation“, Vilmslou, Jungtinė Karalystė), naudojant 0,1 proc. skruzdžių rūgštį kaip užkrovimo buferį, esant 15 µL/min tėkmės greičiui. Peptidai vėliau buvo atskiriami analitinėje HSS-T3 C18 kolonėlėje (1,8 µm dalelių dydis, 75 µm × 250 mm; „Waters Corporation“, Jungtinė Karalystė), taikant linijinį 3–40 proc. tirpiklio B gradientą tirpiklyje A per 90 min. Tirpiklis A buvo 0,1 proc. skruzdžių rūgštis, o tirpiklis B – 100 proc. acetonitrilas su 0,1 proc. skruzdžių rūgštimi. Atskyrimas atliktas esant 300 nl/min. tėkmės greičiui, kolonėlės temperatūrą palaikant 40 °C.

Duomenys surinkti naudojant „Synapt G2“ masių spektrometrą („Waters Corporation“, Vilmslou, Jungtinė Karalystė), sujungtą su „MassLynx 4.1“ programine įranga („Waters Corporation“, Vilmslou, Jungtinė Karalystė), taikant teigiamų jonų režimą ir nuo duomenų priklausomos analizės (DDA) strategiją. MS skenavimai (0,6 s, m/z 350–1350) atlikti kartu su septynių intensyviausių pirmtakinių jonų (2+, 3+ arba 4+ krūvio būsenų) MS/MS analize. MS/MS skenavimai buvo registruojami m/z 50–2000 intervale, vieno skenavimo trukmė – 0,6 s. Po dviejų MS/MS skenavimų pirmtakiniai jonai

buvo dinamiškai eliminuojami 100 s laikotarpiui, taikant 50 mDa masės toleranciją.

Peptidų identifikavimas ir santykinis kiekybinis įvertinimas atlikti naudojant „Progenesis QI for Proteomics“ v4.2 („Nonlinear Dynamics“) kartu su „MASCOT“ paieškos sistema v2.2.07. Pirminiai duomenų failai buvo importuoti į „Progenesis“ programą naudojant numatytuosius parametrus, o MS2 spektrai eksportuoti mgf formatu „MASCOT“ analizei. Duomenų bazės paieška atlikta naudojant „Sus scrofa“ etaloninį proteomą (Uniprot UP000008227, 2023 m. kovo 21 d.), taikant šiuos parametrus: fermento specifiškumas netaikytas, pirmtakinių jonų masės tolerancija – 20 ppm, fragmentų masės tolerancija – 0,3 Da. Paieškos rezultatai buvo pakartotinai importuoti į „Progenesis“ ir susieti su MS1 charakteristikomis kiekybiniam vertinimui atlikti.

2.12. Guminukų su kiaulių kepenų hidrolizatais gamyba

Tyrimo metu buvo sukurtos keturios skirtingos guminukų receptūros (2.12.1 lentelė). Agaro milteliai, išgauti iš dumblių *Gelidium sesquipedale*, buvo įsigyti iš „Rotmanka“ (Gdanskas, Lenkija). Guminukams saldinti naudotas ksilitolis („Natur Hurtig“, Niurnbergas, Vokietija). Citrinų rūgštis buvo įsigyta vietinėje parduotuvėje „Maxima“ (Kaunas, Lietuva).

Siekiant maskuoti kartų hidrolizatų skonį, į vandenį įdėta skystos žaliųjų citrinų kvapiosios medžiagos, pagamintos „Stockmeier Food GmbH“ (Herfordas, Vokietija).

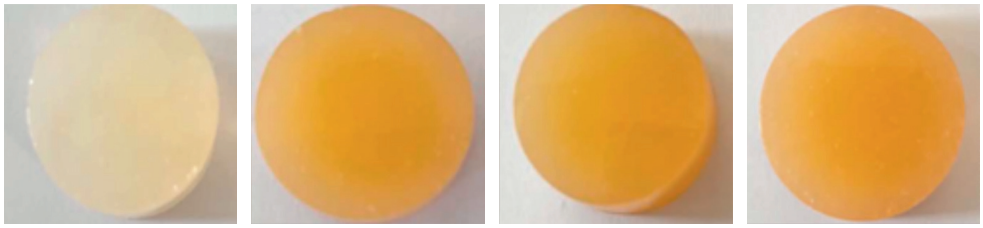
Pirmiausia agaro milteliai buvo tirpinami vandenyje, 3 min. kaitinant 100 °C temperatūroje. Verdant į mišinį buvo dedama cukraus arba ksilitolio, nuolat maišant ir toliau kaitinant. Vėliau į guminukų masę buvo įdedama citrinų rūgšties, hidrolizatų ir kvapiosios medžiagos.

Paruošta masė buvo supilstyta į apvalias silikonines formas (1,9 cm skersmens ir 1,5 cm aukščio). Vieno guminuko masė prieš džiovinimą buvo 2,05 ± 0,18 g, po džiovinimo – 2,01 ± 0,12 g.

2.12.1 lentelė. *Guminukų, papildytų kiaulių kepenų hidrolizatais, sudėtis*

Guminukai	Ag (g)	Vanduo (ml)	Citrinų rūgštis (g)	Ksilitolis (g)	Laimo ekstraktas (ml)	Hidrolizatas (g)
GC	10	100	1	10	0.1	
GC5LPa3Ag	10	100	1	10	0.1	5
GC5LPa6Ag	10	100	1	10	0.1	5
GC5LPa24Ag	10	100	1	10	0.1	5

GC – guminukai; 5,0 – hidrolizatų kiekis (g); 3, 6 ir 24 val. – hidrolizės trukmė; Pa – hidrolizė papainu; Ag – agaras.



GC GC5Pa3Ag GC5Pa6Ag GC5Pa24Ag

2.12.1 pav. *Guminukų, papildytų įvairiais kiaulių kepenų hidrolizatais, išvaizda*

2.13. Guminukų su kiaulių hidrolizatais mikrobiologinis profilis

Atliekant guminukų mėginių mikrobiologinį vertinimą, nustatytas bendras aerobinių mezofilinių bakterijų skaičius, enterobakterijų, mielių ir pelėsių kiekis. Mikrobiologiniai tyrimai atlikti 1, 7 ir 21 laikymo dieną. Guminukai buvo laikomi hermetiškuose polietileniniuose maišeliuose, 18–23 °C temperatūroje, esant 60–65 proc. santykinei oro drėgmei, apsaugant nuo tiesioginių saulės spindulių. Mėginių paruošimas ir nuoseklūs dešimtkarčiai guminukų mėginių praskiedimai buvo atliekami steriliame buferiniame peptoniniame vandenyje pagal ISO 6887-1:2017 [266] ir ISO 6887-4:2017 [267] standartus, nustatančius bendrąsias ir produktui specifines konditerijos gaminių mėginių paruošimo procedūras. Iš kiekvieno praskiedimo po 1 ml mėginio buvo pasėjama dviem pakartojimais mikroorganizmų kiekiui nustatyti. Bendras aerobinių mezofilinių bakterijų skaičius nustatytas pagal ISO 4833-1:2013 [268] standartą „Plate Count Agar“ terpėje (PCA; „Merck“, Vokietija), inkubuojant 30 ± 1 °C temperatūroje 72 ± 3 val. *Enterobacteriaceae* kiekis nustatytas pagal ISO 21528-2:2017 [269] standartą „Violet Red Bile Glucose Agar“ terpėje (VRBG; „Biolife“, Italija), inkubuojant 37 ± 1 °C temperatūroje 24 ± 2 val. Mielių ir pelėsių kiekis vertintas pagal ISO 21527-1:2008 [270] standartą „Dichloran Rose Bengal Chloramphenicol Agar“ terpėje (DRBC; „Liofilchem“,Rozeto delji Abrucis, Italija), inkubuojant 25 ± 1 °C temperatūroje 5 dienas. Rezultatai išreikšti kolonijas formuojančiais vienetais grame mėginio (KSV/g).

2.14. Guminukų maistinės sudėties nustatymas

Guminukų maistinė sudėtis nustatyta standartizuotais metodais, plačiai taikomais atliekant maisto produktų analizę. Baltymų kiekis nustatytas Dumas metodu pagal AOAC 990.03 [259]. Lipidų kiekis nustatytas taikant rūgštinę hidrolizę ir tirpiklinę ekstrakciją pagal AOAC 991.36 [240]. Drėgmės kiekis nustatytas pagal AOAC 925.45 [260] standartą, džiovinant apie 3 g homogenizuoto mėginio iki pastoviosios masės. Pelenų kiekis nustatytas

pagal AOAC 923.03 [260]. Angliavandenių kiekis apskaičiuotas skirtumo metodu, iš bendros masės atimant baltymų, riebalų, drėgmės ir pelenų kiekių sumą.

2.15. Guminukų fizikinių ir cheminių savybių vertinimas

2.15.1. Spalvų koordinatės

Spalvų savybės įvertintos nešiojamuoju kolorimetru „PCE-CSM5“ („PCE Instruments“, Jungtinė Karalystė), taikant CIE L*a*b*C*H* spalvų erdvės sistemą. Šioje sistemoje L* reikšmė nusako šviesumą, a* – žalios ir raudonos spalvų ašį, o b* – mėlynos ir geltonos spalvų ašį.

Spalvos sodrumas išreiškiamas C* (chroma) reikšme, o dominuojantis spektrinis komponentas (raudona, žalia arba mėlyna spalva) apibūdinamas H* (spalvos tono kampo) reikšme, kuri gali svyruoti nuo 0° iki 360°.

2.15.2. Tekstūra

Tekstūra (kietumas) buvo vertinama naudojant tekstūros analizatorių „Brookfield CT-3 Texture Analyser“ („Brookfield Engineering Laboratories“, Middleboro, MA, JAV).

2.15.3. pH nustatymas

Mėginių pH nustatytas pagal EN ISO 2917:2002 [261] standartą. Matavimams naudotas pH matuoklis „Inolab 3“ („Hanna Instruments“, Milanas, Italija). Prieš analizę prietaisas buvo kalibruojamas naudojant standartinius buferinius tirpalus, kurių pH vertės buvo 4,01 ir 7,00 („Sigma-Aldrich“, St. Louis, MO, JAV). Po kalibravimo pH elektrodo zondas buvo tiesiogiai įterpiamas į hidrolizuotų guminukų mėginius.

2.16. Guminukų bendro priimtinumo vertinimas

Pagal ISO 8586-1 juslinio vertinimo metodiką bendrą pagamintų guminukų su kiaulių kepenų hidrolizatais priimtinumą vertino aštuoni apmokyti vertintojai [262].

2.17. Guminukų antioksidacinio aktyvumo nustatymas

2.17.1. DPPH• laisvųjų radikalų surišimo metodas

Standartinis DPPH• radikalų tirpalas buvo paruoštas tiksliai pasvėrus 0,0024 g DPPH (±0,0001 g) ir ištirpinus jį 80 proc. (t/v) etanolyje naudojant ultragarso vonelę. Gautas tirpalas matavimo kolboje buvo praskiestas iki

100 ml tūrio. Tirpalo absorbcija matuota ties 515 nm bangos ilgiu, kaip etaloną naudojant 80 proc. etanolį.

Guminukų ekstraktai buvo paruošti ištirpinus 1 g produkto 100 ml distiliuoto vandens ir nuolat maišant 1 val. kambario temperatūroje. Po to mišinys buvo 10 min. centrifuguojamas 4 °C temperatūroje, esant 8000 aps./min. greičiui. Surinktas supernatantas buvo perfiltruotas per 0,45 μm PTFE membraninį filtrą ir laikomas −80 °C temperatūroje iki tolesnės analizės.

Antioksidacinis aktyvumas vertintas 2,2-difenil-1-pikrilhidrazilio ($DPPH^{\bullet}$) radikalų surišimo metodu, kiekvieną ekstraktą analizuojant trimis pakartojimais. Antiradikalinis aktyvumas nustatytas sumaišius 60 μl ekstrakto su 3 ml 6×10^{-5} koncentracijos etanolinio $DPPH^{\bullet}$ tirpalo 1 cm kiuvetėje. Aktyvumas apskaičiuotas pagal mėginyje esančių antioksidacinių junginių neutralizuotų stabilių $DPPH^{\bullet}$ radikalų dalį. Absorbcijos sumažėjimas ties 515 nm bangos ilgiu buvo stebimas spektrofotometriškai iki pusiausvyros pasiekimo (apie 30 min.). Rezultatai išreikšti inhibuotų $DPPH^{\bullet}$ radikalų procentais.

$$\text{Antioksidacinis aktyvumas } (DPPH^{\bullet}) = \left(\frac{Ab - Aa}{Ab}\right) \cdot 100 \text{ proc.}$$

kur: Ab – kontrolinio mėginio absorbcija (t = 0 min.); Aa – mėginio su tiriamuoju guminukų ekstraktu absorbcija (t = 30 min.).

2.17.2. $ABTS^{\bullet+}$ radikalų katijonų surišimo metodas

Laisvųjų radikalų surišimo geba buvo vertinama spektrofotometriniu metodu. Paruošus 2 mM pradinį $ABTS^{\bullet+}$ tirpalą, jis buvo laikomas tamsaus stiklo butelyje 16 val. Siekiant gauti 0,800 absorbciją ties 734 nm bangos ilgiu, pradinis tirpalas praskiestas distiliuotu vandeniu iki darbui tinkamos $ABTS^{\bullet+}$ tirpalo koncentracijos. Kaip etaloninis tirpalas (blankas) naudotas distiliuotas vanduo.

Guminukų ekstraktai paruošti ištirpinus 1 g produkto 100 ml distiliuoto vandens, nuolat maišant 1 val. kambario temperatūroje. Po to mišinys buvo centrifuguojamas 10 min. 4 °C temperatūroje, esant 8000 aps./min. greičiui. Surinktas supernatantas buvo perfiltruotas per 0,45 μm PTFE membraninį filtrą ir laikomas −80 °C temperatūroje iki tolesnės analizės.

$$\text{Antioksidacinis aktyvumas } (ABTS^{\bullet+}) = \left(\frac{C \cdot V}{m}\right)$$

kur: C – Trolokso koncentracija (μmol/l), nustatyta pagal kalibracinę kreivę; V – ekstrakto tūris (l); m – mėginio masė (g).

2.18. Guminukų antibakterinio aktyvumo nustatymas

Agaro pagrindu pagamintų guminukų antibakterinis aktyvumas buvo vertinamas naudojant tas pačias su maistu susijusių sąlyginai patogeniškų bakterijų etalonines padermes: *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus subsp. aureus* ATCC 25923 ir *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028.

Petri lėkštelės buvo paruoštos naudojant agarinių duobučių difuzijos metodą. Agarui sukietėjus, tiriamieji guminukai (10 × 10 mm) buvo dedami tiesiai ant agaro paviršiaus. Plokštelės inkubuotos 18–24 val. 30 °C temperatūroje (*B. cereus*) ir 37 °C temperatūroje kitoms bakterijų padermėms. Po inkubacijos augimo slopinimo zonos (skaidrios inhibicijos zonos aplink guminukus) buvo matuojamos milimetrais.

Teigiamai kontrolei naudoti guminukai su citrinų rūgštimi ir agaru, o neigiamai kontrolei – sterilus vanduo su agaru.

2.19. Guminukų mikrostruktūrinis įvertinimas

Guminukų mėginių skerspjūviai buvo tiriami naudojant skenuojantį elektroninį mikroskopą „Mira3“ (SEM; „Tescan Orsay Holding, a.s.“, Brno, Čekija). Mėginiai buvo rankiniu būdu supjaustyti į mažus skerspjūvius (0,4 × 0,4 cm) ir dvipuse anglies juosta pritvirtinti prie SEM laikiklių.

Vėliau mėginiai buvo padengti 6 nm storio aukso-paladžio sluoksniu, naudojant vakuuminį dulkinimo įrenginį „Leica EM ACE600“ („Leica Microsystems“, Viena, Austrija). Tyrimui naudoti atspindėtų elektronų (BSE) ir antrinių elektronų (SE) detektoriai. Vaizdai buvo registruojami taikant 1,0 kV didinimą ir 5 kV greitinimo įtampą, siekiant tiksliai apibūdinti mėginių struktūrines savybes ir matmenis.

2.20. Statistinė duomenų analizė

Duomenys buvo suvesti ir kaupiami programoje „Microsoft Excel“. Statistinė analizė atlikta naudojant „IBM SPSS“ programinės įrangos 29.0 versiją („SPSS Inc.“, Čikaga, JAV). Aprašomojoje statistikoje pateikti tiriamųjų grupių rodikliai: vidurkis ir standartinis nuokrypis. Riebalų rūgščių sudėtis išreikšta procentine dalimi nuo bendro identifikuotų riebalų rūgščių kiekio. Duomenų pasiskirstymo normalumas buvo vertinamas taikant Shapiro–Wilk testą, o dispersijų homogeniškumas – Levene testu prieš atliekant dispersinę analizę (ANOVA).

Duomenų analizė atlikta taikant dispersinę analizę (ANOVA). Trijų veiksnių ANOVA buvo taikyta siekiant įvertinti ūkinių gyvūnų rūšių, organų

tipo ir subproduktų grupių (E ir C3) poveikį. Kepenų hidrolizatų antioksidacinis aktyvumas vertintas analizuojant subproduktų grupės, fermento tipo, hidrolizės trukmės ir jų sąveikos poveikį. Statistinis reikšmingumas nustatytas taikant trijų veiksnių ANOVA modelį, po kurio atliktas *Tukey's HSD post hoc* testas ($p < 0,001$).

Dviejų veiksnių ANOVA taikyta hidrolizės laipsniui (DH) analizuoti, vertinant hidrolizės trukmės ir fermento tipo poveikį. Statistiškai reikšmingiems skirtumams nustatyti taikytas *Tukey's HSD post hoc* testas ($p < 0,001$).

Ryšiams tarp antioksidacinio aktyvumo rodiklių ir identifikuotų peptidų santykinės gausos, nustatytos pagal chromatografinių pikų plotus, įvertinti taikytas Pearsono koreliacijos koeficientas ($p < 0,01$).

Statistiniai guminukų duomenys analizuoti taikant vienkryptę ANOVA, o statistiškai reikšmingiems skirtumams tarp mėginių vidurkių nustatyti taikytas *Tukey's HSD post hoc* testas ($p < 0.05$).

3. REZULTATAI

3.1. Galvijų, kiaulių ir avių tyrimas po skerdimo

Veterinarinio tyrimo po skerdimo metu nustatytų patologinių galvijų organų sistemų pokyčių pasiskirstymas pateiktas 3.1.1 lentelėje. Karvių grupėje dažniausiai nustatyti virškinimo sistemos pokyčiai (10,09 proc.), rečiau – širdies ir kraujagyslių sistemos (7,65 proc.), kvėpavimo sistemos (5,50 proc.) ir šlapimo sistemos pokyčiai (5,20 proc.). Bulių grupėje taip pat dominavo virškinimo sistemos pokyčiai (12,35 proc.), po jų – kvėpavimo sistemos (6,79 proc.), šlapimo sistemos (6,17 proc.) ir širdies bei kraujagyslių sistemos pokyčiai (6,17 proc.). Telyčių grupėje didžiausią dalį sudarė virškinimo sistemos pokyčiai (4,50 proc.), kvėpavimo bei širdies ir kraujagyslių sistemos pokyčiai nustatyti rečiau (2,70 proc.), o šlapimo sistemos pokyčių nenustatyta. Tarp galvijų kategorijų nenustatyta nė vienos tirtos organų sistemos statistiškai reikšmingų skirtumų: šlapimo sistemos pokyčių ($\chi^2 = 3,72$; $p = 0,15$), virškinimo sistemos pokyčių ($\chi^2 = 2,56$; $p = 0,28$), kvėpavimo sistemos pokyčių ($\chi^2 = 1,34$; $p = 0,51$) bei širdies ir kraujagyslių sistemos pokyčių ($\chi^2 = 0,96$; $p = 0,62$).

3.1.1 lentelė. *Veterinarinio tyrimo po skerdimo metu nustatyti patologiniai galvijų organų sistemų pokyčiai*

Organų sistema	Karvės (n = 327), n (proc.)	Buliai (n = 162), n (proc.)	Telyčios (n = 111), n (proc.)	χ^2	p
Kvėpavimo sistemos pokyčiai	18 (5,50)	11 (6,79)	3 (2,70)	1,34	0,51
Virškinimo sistemos pokyčiai	33 (10,09)	20 (12,35)	5 (4,50)	2,56	0,28
Šlapimo sistemos pokyčiai	17 (5,20)	10 (6,17)	N.D.	3,72	0,15
Širdies ir kraujagyslių sistemos pokyčiai	25 (7,65)	10 (6,17)	3 (2,70)	0,96	0,62

Duomenys pateikti kaip patologinių pokyčių skaičius ir procentinė dalis, n (proc.). Procentinė galvijams nustatytų patologinių pokyčių dalis apskaičiuota kiekvienoje galvijų grupėje. N.D. – nenustatyta.

Veterinarinio tyrimo po skerdimo metu galvijams nustatyti patologiniai pokyčiai apibendrinti 3.1.2 lentelėje. Tarp kepenų pokyčių dažniausiai nustatyta fascioliozė (3,33 proc.), rečiau – kepenų riebalinė degeneracija (2,17 proc.) ir lėtinis cholangitas (1,67 proc.). Tarp kvėpavimo sistemos pokyčių dažniausiai nustatyta plaučių emfizema (2,33 proc.) ir plaučių aspiracija (2 proc.).

3.1.2 lentelė. *Veterinarinio tyrimo po skerdimo metu galvijams nustatyti patologiniai pokyčiai*

Pokyčiai	Karvės (n = 327), n (proc.)*	Buliai (n = 162), n (proc.)*	Telyčios (n = 111), n (proc.)*	Iš viso (n)	Iš viso (proc.) *
Riebalinė kepenų degeneracija	8 (1,33)	5 (0,83)	N.D.	13	2,17
Kepenų abscesai	3 (0,50)	2 (0,33)	1 (0,17)	6	1,00
Kepenų fascioliozė	12 (2,00)	5 (0,83)	3 (0,50)	20	3,33
Lėtinis cholangitas	5 (0,83)	4 (0,67)	1 (0,17)	10	1,67
Kepenų hiperemija	5 (0,83)	3 (0,50)	N.D.	8	1,33
Židininė kepenų nekrozė	N.D.	1 (0,17)	N.D.	1	0,17
Fibrinis perikarditas	6 (1,00)	8 (1,33)	3 (0,50)	17	2,83
Endokarditas	9 (1,50)	2 (0,33)	N.D.	11	1,83
Miokardo hemoragijos	10 (1,67)	9 (1,50)	1 (0,17)	20	3,33
Plaučių emfizema	9 (1,50)	5 (0,83)	3 (0,50)	14	2,33
Aspiracinė pneumonija	6 (1,00)	6 (1,00)	N.D.	12	2,00

3.1.2 lentelės tęsinys

Pokyčiai	Karvės (n = 327), n (proc.)*	Buliai (n = 162), n (proc.)*	Telyčios (n = 111), n (proc.)*	Iš viso (n)	Iš viso (proc.) *
Pneumonija	3 (0,50)	N.D.	N.D.	3	0,50
Lėtinis intersticinis nefritas	9 (1,50)	9 (1,50)	N.D.	18	3,00
Inkstų cistos	8 (1,33)	1 (0,17)	N.D.	9	1,50
Bendras patologinių pokyčių skaičius				162	27,00

Visų patologinių pokyčių procentinė dalis apskaičiuota nuo bendro tirtų galvijų skaičiaus. N.D. – nenustatyta.

Veterinarinio tyrimo po skerdimo metu kiaulėms nustatyti patologiniai pokyčiai pagal organų sistemas pateikti 3.1.3 lentelėje. Iš viso patologiniai pokyčiai nustatyti 81 kiaulių skerdenoje (13,50 proc.), o likusiose 519 skerdenų (86,50 proc.) patologinių pokyčių nenustatyta. Dažniausiai registruoti širdies ir kraujagyslių sistemos pokyčiai, nustatyti 4,83 proc. visų tirtų skerdenų. Inkstų pokyčiai nustatyti 20 atvejų (3,33 proc.), o kvėpavimo sistemos bei širdies ir kraujagyslių sistemos pokyčiai buvo vienodai dažni – po 17 atvejų (2,83 proc.).

3.1.3 lentelė. *Veterinarinio tyrimo po skerdimo metu nustatyti patologiniai kiaulių organų sistemų pokyčiai*

Organų sistema	Kiaulės (n = 600), n (proc.)*
Kvėpavimo sistemos pokyčiai	17 (2,83)
Virškinimo sistemos pokyčiai	27 (4,50)
Šlapimo sistemos pokyčiai	20 (3,33)
Širdies ir kraujagyslių sistemos pokyčiai	29 (4,83)

*Procentinė pokyčių dalis apskaičiuota nuo bendro tirtų kiaulių skaičiaus.

Veterinarinio tyrimo po skerdimo metu kiaulėms nustatyti patologiniai pokyčiai apibendrinti 3.1.3 lentelėje. Išsamesnė rezultatų analizė parodė, kad dažniausiai nustatytos inkstų cistos – jos rastos 20 ūkinių gyvūnų (3,33 proc.).

Kepenų patologiniai pokyčiai daugiausia pasireiškė kepenų riebaline degeneracija (13 atvejų; 2,17 proc.) ir kepenų nekroze (11 atvejų; 1,83 proc.), o kepenų abscesai retesni (3 atvejai; 0,50 proc.). Tiriant kvėpavimo sistemą, dažniausiai diagnozuota lėtinė bronchopneumonija (9 atvejai; 1,50 proc.), po jos sekė aspiracinė pneumonija (8 atvejai; 1,33 proc.) ir plaučių emfizema (9 atvejai; 1,50 proc.). Dažniausi širdies ir kraujagyslių sistemos pokyčiai

buvo miokardo hemoragijos (12 atvejų; 2,00 proc.), po jų – endokarditas (10 atvejų; 1,67 proc.) ir fibrininis perikarditas (7 atvejai; 1,17 proc.).

3.1.4 lentelė. *Veterinarinio tyrimo po skerdimo metu kiaulėms nustatyti patologiniai pokyčiai*

Pokyčiai	Kiaulės (n = 600), n (proc.)*
Riebalinė kepenų degeneracija	13 (2,17)
Kepenų nekrozė	11 (1,83)
Kepenų abscesai	3 (0,50)
Fibrinis perikarditas	7 (1,17)
Endokarditas	10 (1,67)
Miokardo hemoragijos	12 (2,00)
Lėtinė bronchopneumonija	9 (1,50)
Aspiracinė bronchopneumonija	8 (1,33)
Plaučių emfizema	9 (1,50)
Inkstų cistos	20 (3,33)
Bendras patologinių pokyčių skaičius	93 (15,50)

*Procentinė pokyčių dalis apskaičiuota nuo bendro tirtų kiaulių skaičiaus.

Veterinarinio tyrimo po skerdimo metu avims nustatytų patologinių pokyčių pasiskirstymas organų sistemose pateiktas 3.1.4 lentelėje. Atlikus 600 avių skerdenų veterinarinį tyrimą po skerdimo, patologiniai pokyčiai nustatyti 61 skerdenoje (10,17 proc.), o likusiose 539 skerdenose (89,83 proc.) patologinių pokyčių nenustatyta.

Dažniausiai registruoti kepenų pokyčiai, nustatyti 33 skerdenose, sudarantys 5,50 proc. visų tirtų gyvūnų. Šlapimo sistemos pažeidimai nustatyti 10 atvejų (1,67 proc.), o kvėpavimo bei širdies ir kraujagyslių sistemos pokyčiai registruoti vienodai dažnai – po 9 atvejus kiekvienoje grupėje (1,50 proc.).

3.1.5 lentelė. *Veterinarinio tyrimo po skerdimo metu nustatyti patologiniai avių organų sistemų pokyčiai*

Organų sistema	Avys (n = 600), n (proc.)*
Kvėpavimo sistemos pokyčiai	9 (1,50)
Virškinimo sistemos pokyčiai	33 (5,50)
Šlapimo sistemos pokyčiai	10 (1,67)

*Procentinė patologinių pokyčių dalis apskaičiuota nuo bendro tirtų avių skaičiaus.

Veterinarinio tyrimo po skerdimo metu avims nustatyti patologiniai pokyčiai apibendrinti 3.1.6 lentelėje. Išsamesnė rezultatų analizė parodė, kad dažniausias kepenų patologinis pokytis – kepenų fibrozė, nustatyta 14 gyvūnų (2,33 proc.). Rečiau nustatyta kepenų riebalinė degeneracija – 9 atvejai (1,50 proc.) ir kepenų abscesai – 8 atvejai (1,33 proc.), o kepenų fascioliozė diagnozuota tik 2 gyvūnams (0,33 proc.).

Vienintelis nustatytas kvėpavimo sistemos patologinis pokytis buvo plaučių emfizema, diagnozuota 9 skerdenose (1,50 proc.). Širdies ir kraujagyslių sistemos patologiniams pokyčiams priskirtos miokardo hemoragijos, nustatytos 9 gyvūnams (1,50 proc.). Tarp šlapimo sistemos patologinių pokyčių dažniau nustatytos inkstų cistos (8 atvejai; 1,33 proc.), o nefritas diagnozuotas tik 2 gyvūnams (0,33 proc.).

3.1.6 lentelė. *Veterinarinio tyrimo po skerdimo metu avims nustatyti patologiniai pokyčiai*

Pokyčiai	Avys (n = 600), n (proc.)*
Kepenų fibrozė	14 (2,33)
Kepenų fascioliozė	2 (0,33)
Kepenų riebalinė degeneracija	9 (1,50)
Kepenų abscesai	8 (1,33)
Plaučių emfizema	9 (1,50)
Miokardo hemoragijos	9 (1,50)
Nefritas	2 (0,33)
Inkstų cistos	8 (1,33)
Bendras patologinių pokyčių skaičius	61 (10,17)

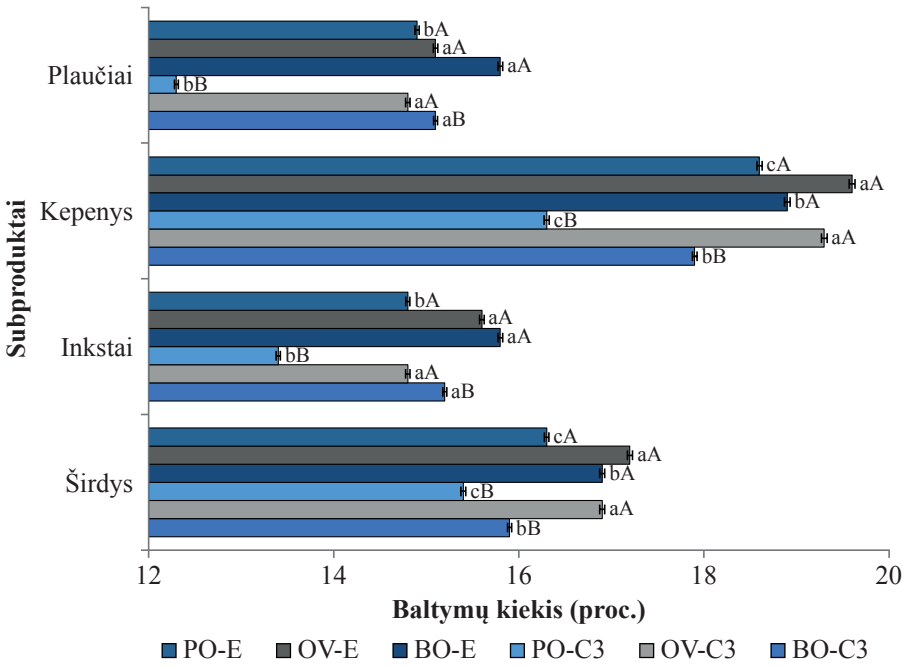
*Procentinė patologinių pokyčių dalis apskaičiuota nuo bendro tirtų avių skaičiaus.

3.2. Liofilizuotų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų maistinės sudėties įvertinimas

3.2.1. Baltymai

Liofilizuotų valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų baltymų kiekis pateiktas 3.2.1.1 pav. Nustatyta, kad baltymų kiekiui statistiškai reikšmingą įtaką turėjo organo tipas, gyvulių rūšis ir subprodukto grupė (E ir C3) ($p < 0,05$). Didžiausias baltymų kiekis nustatytas kepenų mėginiuose (16,3–19,3 ± 0,01 proc.), širdies mėginiuose taip pat fiksuotas didelis kiekis baltymų (15,4 ± 0,01–17,2 ± 0,02 proc.), o mažiausias – plaučių mėginiuose (12,3 ± 0,02–15,8 ± 0,02 proc.). Tame pačiame subprodukte ir subprodukto grupėje tarp gyvūnų rūšių nustatyti reikšmingi baltymų kiekio

skirtumai ($p < 0,05$). Didžiausi skirtumai nustatyti kepenyse ir inkstuose, kuriuose avių valgomuosiuose subproduktuose baltymų kiekis buvo didesnis nei galvijų ir kiaulių. Taip pat nustatyti reikšmingi skirtumai tarp valgomųjų subproduktų ir 3 kategorijos šalutinių produktų, vertinant skirtingų ūkinių gyvūnų rūšių to paties subprodukto mėginius ($p < 0,05$). Visuose tirtuose organuose valgomųjų subproduktų (E) mėginiuose baltymų kiekis buvo statistiškai reikšmingai didesnis nei 3 kategorijos (C3) šalutiniuose gyvūniniuose produktuose.



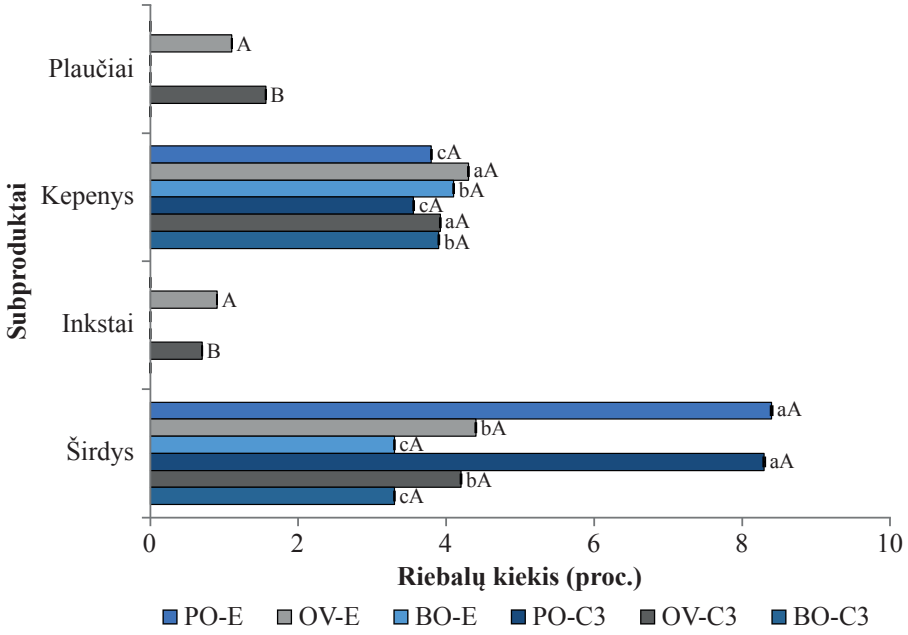
3.2.1.1 pav. *Liofilizuotų valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų baltymų kiekis*

Skirtingos mažosios raidės (a–c) prie to paties subprodukto ir subproduktų grupės rodo statistiškai reikšmingus skirtumus tarp rūšių ($p < 0,05$). Skirtingos didžiosios raidės (A–B) prie to paties subprodukto ir ūkinio gyvūno rūšies rodo statistiškai reikšmingus skirtumus tarp valgomųjų subproduktų ir 3 kategorijos gyvūninių šalutinių produktų grupių ($p < 0,05$). Vertinant rezultatus, analizuojami statistiškai reikšmingi skirtumai tarp valgomųjų subproduktų (E) ir 3 kategorijos gyvūninių šalutinių produktų (C3). PO – kiaulė; OV – avis; BO – galvijas; E – valgomieji subproduktai; C3 – 3 kategorijos šalutiniai gyvūniniai produktai pagal Reglamentą (EB) Nr. 1069/2009.

3.2.2. Riebalai

Liofilizuotų valgomųjų subproduktų ir 3 kategorijos gyvūninių šalutinių produktų riebalų kiekis pateiktas 3.2.2.1 pav. Nustatyta, kad riebalų kiekiui statistiškai reikšmingą įtaką turėjo subprodukto tipas ir gyvulio rūšis ($p < 0,05$). Didžiausias riebalų kiekis nustatytas kiaulių širdyse (8,4 ± 0,02 proc.) ir avių kepenyse (5,2 ± 0,01 proc.), o tai rodo reikšmingus skirtumus tarp ūkinių gyvūnų rūšių ir subproduktų tipų.

Palyginus valgomuosius subproduktus su 3 kategorijos gyvūniniais šalutiniais produktais, nustatyta, kad plaučių ir inkstų mėginiuose riebalų kiekis reikšmingai skyrėsi ($p < 0,05$), o kepenų ir širdies mėginiuose statistiškai reikšmingų skirtumų tarp šių subproduktų grupių nenustatyta.



3.2.2.1 pav. *Liofilizuotų valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų baltymų kiekis*

Skirtingos mažosios raidės (a–c) prie to paties subprodukto ir subproduktų grupės rodo statistiškai reikšmingus skirtumus tarp rūšių ($p < 0,05$). Skirtingos didžiosios raidės (A–B) prie to paties subprodukto ir gyvūnų rūšies rodo statistiškai reikšmingus skirtumus tarp valgomųjų subproduktų ir 3 kategorijos gyvūninių šalutinių produktų grupių ($p < 0,05$). Vertinant rezultatus, analizuojami statistiškai reikšmingi skirtumai tarp valgomųjų subproduktų (E) ir 3 kategorijos šalutinių gyvūninių produktų (C3). PO – kiaulė; OV – avis; BO – galvijas; E – valgomieji subproduktai; C3 – 3 kategorijos šalutiniai gyvūniniai produktai pagal Reglamentą (EB) Nr. 1069/2009.

3.2.3. Riebalų rūgščių sudėtis ir lipidų kokybės indeksai

Valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų riebalų rūgščių (RR) grupės ir lipidų kokybės indeksai (LQI) pateikti 3.2.3.1 lentelėje. Gyvūnų plaučių mėginiuose sočiosios riebalų rūgštys (SRR) sudarė didžiausią bendro riebalų rūgščių kiekio dalį ir svyravo nuo 53,85 proc. iki 56,20 proc. valgomųjų subproduktų mėginiuose (E) bei nuo 56,55 proc. iki 58,87 proc. 3 kategorijos šalutinių produktų mėginiuose (C3) ($p < 0,05$). Polinesočiųjų riebalų rūgščių (PNRR) kiekis buvo didžiausias kiaulių plaučiuose ($23,79 \pm 0,05$ proc.) ir mažiausias avių plaučiuose ($11,90 \pm 0,05$ proc.) ($p < 0,05$). 3 kategorijos plaučių mėginiams buvo būdingas didesnis omega-6 / omega-3 santykis (iki 14,57) ir didesni aterogeniškumo (AI) bei trombogeniškumo (TI) indeksai (iki 2,47), palyginti su valgomaisiais mėginiais ($p < 0,05$).

Kepenų mėginiuose SRR taip pat sudarė didžiausią visų riebalų rūgščių dalį ir valgomuosiuose subproduktuose svyravo nuo 50,70 proc. iki 56,76 proc., o C3 mėginiuose – nuo 50,72 iki 53,14 proc. ($p < 0,05$). PNRR kiekis tarp rūšių reikšmingai skyrėsi ($p < 0,05$) ir buvo didžiausias kiaulių kepenyse ($26,69 \pm 0,05$ proc.), o avių ir galvijų kepenyse mažesnis (atitinkamai 12,11 proc. ir 10,20 proc.). Didžiausias omega-6 riebalų rūgščių kiekis buvo kiaulių kepenyse ($23,46 \pm 0,04$ proc.), o avių mėginiuose išliko reikšmingai mažesnis (5,13 proc.) ($p < 0,05$).

C3 kepenų mėginiuose PNRR kiekis (24,16–28,03 proc.) ir PNRR/SRR santykis (0,46–0,55) paprastai buvo didesni, palyginti su valgomųjų subproduktų mėginiais ($p < 0,05$). C3 kepenyse nustatytas didesnis h/H santykis (2,06–2,84) nei valgomuosiuose subproduktuose (0,47–1,32) ($p < 0,05$), o tai rodo lipidų kokybės skirtumus tarp subproduktų grupių.

Inkstų mėginiuose SRR sudarė didžiausią visų riebalų rūgščių dalį ir svyravo nuo 46,53 iki 60,89 proc. valgomuosiuose subproduktuose ir nuo 57,10 proc. iki 61,32 proc. C3 mėginiuose ($p < 0,05$). Polinesočiųjų riebalų rūgščių kiekis reikšmingai skyrėsi tarp rūšių ($p < 0,05$). Didžiausias jų kiekis nustatytas galvijų inkstuose ($25,71 \pm 0,03$ proc.), o kiaulių ir avių inkstuose – mažesnis. Galvijų inkstų mėginiuose taip pat nustatytas reikšmingai didesnis omega-3 riebalų rūgščių kiekis ($17,93 \pm 0,05$ proc.), o kiaulių ir avių mėginiuose – reikšmingai mažesnis ($p < 0,05$). Galvijų inkstų mėginiuose nustatytas palankesnis PNRR/SRR santykis (0,55) ir mažesnis trombogeniškumo indeksas (0,59), palyginti su kiaulių ir avių inkstais ($p < 0,05$). C3 inkstų mėginiuose PNRR kiekis svyravo nuo 15,02 iki 20,72 proc., o PNRR/SRR santykis – nuo 0,26 iki 0,34. h/H santykis buvo reikšmingai didesnis C3 mėginiuose (1,48–1,83) nei valgomuosiuose subproduktuose (0,46–1,18) ($p < 0,05$), kas patvirtina lipidų kokybės skirtumus tarp šių produktų subproduktų grupių.

3.2.3.1 lentelė. *Liofilizuotų valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų riebalų rūgščių (RR) kiekiai grupėse ir lipidų kokybės indeksai*

Mėginys		SRR	MRR	PRR	NRR	Omega-3	Omega-6	Omega-6/3	PRR/SRR	AI	TI	h/H
Plaučiai	Lu-PO-E	$54,84^{bB}$	$21,46^{cB}$	$23,79^{aB}$	$45,25^{bB}$	$5,63^{bB}$	$18,16^{aB}$	$3,22^{bB}$	$0,43^{aB}$	$0,73^{bB}$	$1,47^{bB}$	$0,92^{aB}$
	Lu-OV-E	$56,20^{aB}$	$31,98^{aB}$	$11,90^{cB}$	$43,88^{cB}$	$6,54^{aB}$	$5,36^{cB}$	$0,82^{cB}$	$0,21^{cB}$	$0,64^{cB}$	$1,37^{cB}$	$0,50^{cB}$
	Lu-BO-E	$53,85^{cB}$	$28,05^{bB}$	$18,10^{bB}$	$46,15^{aB}$	$3,64^{cB}$	$14,46^{bB}$	$3,97^{aB}$	$0,34^{bB}$	$0,67^{aB}$	$1,62^{aB}$	$0,74^{bB}$
	Lu-PO-C3	$58,87^{aA}$	$25,11^{bA}$	$16,02^{cA}$	$41,13^{cA}$	$1,19^{cA}$	$14,83^{bA}$	$12,46^{bA}$	$0,27^{bA}$	$0,91^{aA}$	$2,47^{aA}$	$1,42^{aA}$
	Lu-OV-C3	$57,35^{bA}$	$25,33^{bA}$	$17,32^{bA}$	$42,65^{bA}$	$3,10^{aA}$	$14,12^{bA}$	$4,55^{cA}$	$0,30^{aA}$	$0,80^{bA}$	$1,96^{cA}$	$1,60^{aA}$
	Lu-BO-C3	$56,55^{cA}$	$25,11^{bA}$	$18,53^{aA}$	$43,64^{aA}$	$1,19^{cA}$	$17,34^{aA}$	$14,57^{aA}$	$0,33^{aA}$	$0,81^{bA}$	$2,26^{bA}$	$1,58^{aA}$
	Reikšmė ± SD	0,04	0,04	0,05	0,05	0,05	0,05	0,05	0,04	0,05	0,05	0,05
Kepenys	Li-PO-E	$50,70^{cB}$	$22,62^{cB}$	$26,69^{aB}$	$49,30^{aB}$	$3,22^{bB}$	$23,46^{aB}$	$7,28^{aB}$	$0,53^{aB}$	$0,43^{bB}$	$1,51^{bB}$	$1,32^{bB}$
	Li-OV-E	$56,76^{aB}$	$31,11^{bB}$	$12,11^{bB}$	$43,23^{cB}$	$6,99^{aB}$	$5,13^{cB}$	$0,73^{cB}$	$0,21^{bB}$	$0,52^{aB}$	$1,24^{cB}$	$0,60^{cB}$
	Li-BO-E	$55,99^{bB}$	$33,82^{aB}$	$10,20^{cB}$	$44,01^{bB}$	$2,22^{cB}$	$7,97^{bB}$	$3,59^{bB}$	$0,18^{cB}$	$0,55^{aB}$	$1,96^{aB}$	$0,47^{cB}$
	Li-PO-C3	$53,14^{aA}$	$21,43^{bA}$	$25,43^{bA}$	$46,86^{cA}$	$4,22^{bA}$	$21,21^{aA}$	$5,03^{bA}$	$0,48^{bA}$	$0,47^{bA}$	$1,52^{bA}$	$2,25^{bA}$
	Li-OV-C3	$50,72^{cA}$	$21,24^{bA}$	$28,03^{aA}$	$49,27^{aA}$	$7,68^{aA}$	$20,35^{cA}$	$2,65^{cA}$	$0,55^{aA}$	$0,44^{cA}$	$1,09^{cA}$	$2,84^{aA}$
	Li-BO-C3	$52,50^{bA}$	$23,43^{aA}$	$24,16^{bA}$	$47,59^{cA}$	$3,23^{bA}$	$20,93^{bA}$	$6,48^{aA}$	$0,46^{cA}$	$0,50^{aA}$	$1,61^{aA}$	$2,06^{cA}$
	Reikšmė ± SD	0,05	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Inkstai	K-PO-E	$60,89^{aB}$	$28,76^{bB}$	$10,53^{bB}$	$39,29^{cB}$	$3,46^{cB}$	$7,08^{bB}$	$2,05^{bB}$	$0,17^{cB}$	$0,70^{bB}$	$1,95^{bB}$	$0,46^{cB}$
	K-OV-E	$60,89^{aB}$	$29,40^{aB}$	$9,50^{cB}$	$38,90^{bB}$	$4,43^{bB}$	$5,08^{cB}$	$1,15^{cB}$	$0,16^{cB}$	$0,71^{aB}$	$1,78^{bB}$	$0,41^{cB}$
	K-BO-E	$46,53^{bB}$	$27,75^{cB}$	$25,71^{aB}$	$53,47^{aB}$	$17,93^{aB}$	$7,78^{aB}$	$0,43^{aB}$	$0,55^{aB}$	$0,44^{cB}$	$0,59^{cB}$	$1,18^{bB}$
	K-PO-C3	$57,10^{cA}$	$27,88^{aA}$	$15,02^{bA}$	$42,90^{bA}$	$1,06^{bA}$	$13,96^{bA}$	$13,13^{aA}$	$0,26^{bA}$	$0,67^{bA}$	$2,31^{aA}$	$1,66^{aA}$
	K-OV-C3	$61,32^{aA}$	$18,03^{cA}$	$20,72^{cA}$	$38,75^{cA}$	$4,47^{bA}$	$16,25^{aA}$	$3,64^{cA}$	$0,34^{bA}$	$0,65^{cA}$	$1,82^{bA}$	$1,83^{aA}$
	K-BO-C3	$59,57^{bA}$	$25,22^{bA}$	$15,24^{aA}$	$40,46^{aA}$	$1,41^{aA}$	$13,83^{cA}$	$9,81^{bA}$	$0,26^{bA}$	$0,74^{aA}$	$2,44^{aA}$	$1,48^{aA}$
	Reikšmė ± SD	0,03	0,02	0,02	0,04	0,03	0,03	0,03	0,03	0,04	0,04	0,04

3.2.3.1 lentelės tęsinys

Mėginys		SRR	MRR	PRR	NRR	Omega-3	Omega-6	Omega-6/3	PRR/SRR	AI	TI	h/H
Širdys	H-PO-E	$41{,}11^{cB}$	$42{,}23^{aB}$	$17{,}10^{bB}$	$59{,}32^{aB}$	$1{,}02^{bB}$	$16{,}08^{aB}$	$15{,}77^{aB}$	$0{,}42^{aB}$	$0{,}45^{bB}$	$1{,}26^{bB}$	$0{,}73^{aB}$
	H-OV-E	$62{,}18^{aB}$	$31{,}19^{bB}$	$7{,}50^{cB}$	$38{,}69^{cB}$	$2{,}37^{cB}$	$5{,}13^{cB}$	$2{,}17^{cB}$	$0{,}12^{cB}$	$0{,}97^{aB}$	$2{,}35^{cB}$	$0{,}27^{cB}$
	H-BO-E	$59{,}82^{bB}$	$30{,}62^{cB}$	$9{,}56^{aB}$	$40{,}18^{bB}$	$3{,}05^{bB}$	$6{,}51^{bB}$	$2{,}13^{bB}$	$0{,}16^{bB}$	$0{,}86^{bB}$	$2{,}07^{aB}$	$0{,}34^{bB}$
	H-PO-C3	$57{,}04^{bA}$	$26{,}38^{bA}$	$16{,}67^{bA}$	$43{,}05^{bA}$	$1{,}07^{bA}$	$15{,}60^{bA}$	$14{,}58^{bA}$	$0{,}29^{bA}$	$0{,}84^{bA}$	$2{,}33^{bA}$	$1{,}61^{bA}$
	H-OV-C3	$58{,}66^{aA}$	$20{,}24^{cA}$	$21{,}15^{cA}$	$41{,}39^{cA}$	$4{,}71^{aA}$	$16{,}44^{aA}$	$3{,}49^{cA}$	$0{,}36^{aA}$	$0{,}76^{cA}$	$1{,}88^{cA}$	$1{,}81^{aA}$
	H-BO-C3	$57{,}89^{cA}$	$27{,}58^{aA}$	$14{,}53^{aA}$	$42{,}11^{aA}$	$0{,}92^{cA}$	$13{,}61^{cA}$	$14{,}79^{aA}$	$0{,}25^{cA}$	$0{,}85^{aA}$	$2{,}45^{aA}$	$1{,}43^{cA}$
	Reikšmė ± SD	0,04	0,04	0,02	0,02	0,03	0,03	0,03	0,03	0,04	0,04	0,04

Skirtingos to paties subprodukto ir grupės mažosios raidės ($^{a–c}$) rodo statistiškai reikšmingus skirtumus tarp rūšių ($p < 0{,}05$). Skirtingos to paties subprodukto ir ūkinio gyvūno rūšies didžiosios raidės ($^{A–B}$) rodo statistiškai reikšmingus skirtumus tarp valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų ($p < 0{,}05$). Vertinant rezultatus, analizuojami statistiškai reikšmingi skirtumai tarp valgomųjų subproduktų (E) ir 3 kategorijos gyvūninių šalutinių produktų (C3). Lu – plaučiai (angl. *lungs*); Li – kepenys (angl. *liver*); K – inkstai (angl. *kidney*); H – širdys (angl. *heart*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos šalutiniai gyvūniniai produktai pagal Reglamentą (EB) Nr. 1069/2009.

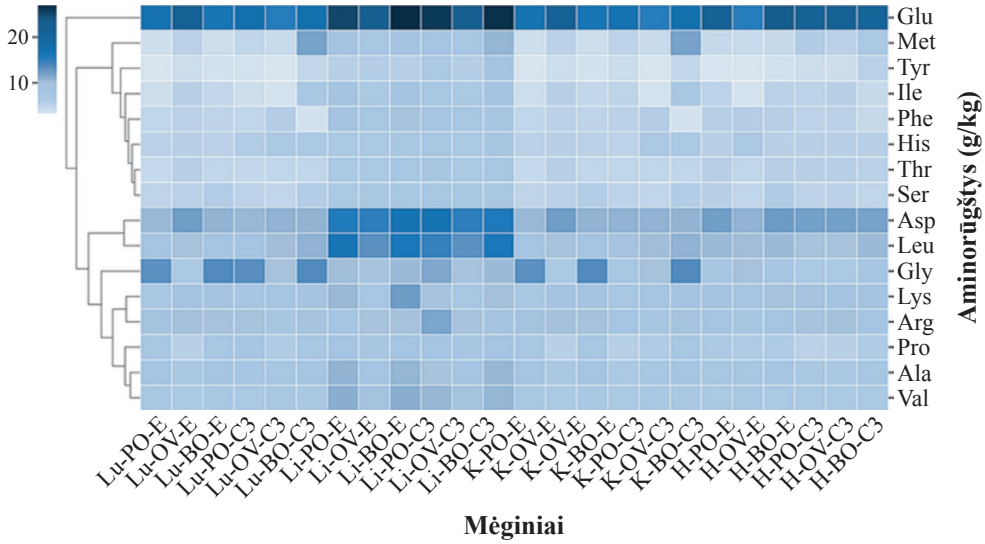
Širdies mėginiuose didžiausią visų riebalų rūgščių dalį sudarė sočiosios riebalų rūgštys (SRR), kurių kiekis valgomuosiuose subproduktuose svyravo nuo 41,11 proc. iki 62,18 proc., o 3 kategorijos (C3) mėginiuose – nuo 57,04 proc. iki 58,66 proc. ($p < 0,05$). Polinesočiųjų riebalų rūgščių (PNRR) kiekis reikšmingai skyrėsi tarp rūšių ($p < 0,05$). Didžiausias jis buvo avių C3 mėginiuose (21,15 ± 0,06 proc.), o mažiausias – avių valgomuosiuose subproduktuose (7,50 ± 0,02 proc.).

Kiaulių širdžių mėginiai pasižymėjo didesniu omega-6 riebalų rūgščių kiekiu ir didesniu omega-6/omega-3 santykiu (iki 15,77), o avių ir galvijų mėginiuose šis santykis buvo reikšmingai mažesnis ($p < 0,05$). Lipidų kokybės rodikliai taip pat reikšmingai skyrėsi – didesni aterogeniškumo (AI) ir trombogeniškumo (TI) indeksai nustatyti avių ir galvijų mėginiuose, o kiaulių mėginiai pasižymėjo palankesniu lipidų profiliu. PNRR/SRR santykis buvo didžiausias kiaulių valgomuosiuose mėginiuose (0,42), o mažiausias – avių mėginiuose (0,12).

Didesni h/H santykiai (1,43–1,81) nustatyti C3 mėginiuose, palyginti su valgomaisiais subproduktais (0,27–0,73) ($p < 0,05$), o tai rodo skirtumus tarp subproduktų grupių.

3.2.4. Aminorūgščių kiekis

Aminorūgščių profiliai liofilizuotuose valgomųjų subproduktų ir 3 kategorijos gyvūninių šalutinių produktų mėginiuose įvertinti naudojant 3.2.4.1 pav. pateiktą šilumos žemėlapio vizualizaciją. Šilumos žemėlapis atskleidė valgomųjų subproduktų ir 3 kategorijos gyvūninių šalutinių produktų aminorūgščių profilių skirtumus. Visų tirtų gyvūnų rūšių valgomiesiems subproduktams ir 3 kategorijai priskirtiems šalutiniams gyvūniniams produktams gausiausios aminorūgštys buvo glutamo rūgštis (Glu), asparto rūgštis (Asp), alaninas (Ala), glicinas (Gly) ir leucinas (Leu), o aromatinės aminorūgštys fenilalaninas ir tirozinas buvo mažesnės koncentracijos ir tolygiau pasiskirsčiusios. Hierarchinė klasterizacija pagal aminorūgščių koncentracijas parodė, kad mėginius pirmiausia galima grupuoti pagal subprodukto tipą, o ne pagal gyvulio rūšį ar subprodukto grupę (valgomieji subproduktai ir 3 kategorijos šalutiniai produktai). Kepenų mėginiai sudarė aiškiai išreikštą klasterį, kuriam būdingos didesnės bendros aminorūgščių koncentracijos, ypač glutamo rūgšties, asparto rūgšties, leucino ir alanino. Antrą pagrindinį klasterį sudarė širdies, inkstų ir plaučių mėginiai, kurie toliau skaidėsi į būdingus pogrupius. Plaučių mėginiai sudarė atskirą pogrupį, atspindintį didesnes glicino ir alanino koncentracijas, o širdies ir inkstų mėginiai buvo artimesni tarpusavyje ir pasižymėjo vidutinėmis aminorūgščių koncentracijomis.



3.2.4.1 pav. *Liofilizuotų valgomųjų subproduktų ir 3 kategorijos gyvūninės kilmės šalutinių produktų aminorūgščių profiliai skirtingose mėginių grupėse (g/kg sausosios medžiagos)*

Lu – plaučiai (angl. *lungs*); Li – kepenys (angl. *liver*); K – inkstai (angl. *kidney*); H – širdys (angl. *heart*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos gyvūninės kilmės šalutiniai produktai.

Trijų veiksnių dispersinė analizė parodė, kad subprodukto tipas buvo pagrindinis veiksnys, lemiantis visų tirtų aminorūgščių koncentracijų skirtumus ($p < 0,001$). Glutamo rūgštis buvo dominuojanti aminorūgštis visuose mėginiuose, nepriklausomai nuo gyvulio rūšies, subprodukto tipo ar subproduktų grupės. Tačiau jos koncentracijai statistiškai reikšmingą įtaką turėjo subprodukto tipas ($F = 329,45$; $p < 0,001$).

Nepakeičiamųjų aminorūgščių koncentracijos taip pat reikšmingai skyrėsi, priklausomai nuo subprodukto tipo, kuris buvo pagrindinis veiksnys, nulemiantis visų tirtų nepakeičiamųjų aminorūgščių pasiskirstymą ($p < 0,001$). Didžiausios bendros šių aminorūgščių koncentracijos nustatytos kepenų mėginiuose, reikšmingai viršijusios širdies ir plaučių vertes. Tarp nepakeičiamųjų aminorūgščių visuose tirtuose subproduktuose dominavo leucinas, kurio koncentracija buvo didžiausia kepenyse ir žymiai mažesnė širdyse bei plaučiuose ($p < 0,001$). Metioninas išsiskyrė ryškiausiais skirtumais tarp subproduktų: didžiausios jo koncentracijos nustatytos kepenyse, o mažiausios – širdžių mėginiuose ($p < 0,001$). Treonino koncentracija taip pat reikšmingai priklausė nuo subproduktų tipo ($p < 0,001$): didesnės koncentracijos nustatytos kepenų ir inkstų mėginiuose, o mažiausios – plaučiuose. Valino

koncentracija mažiau skyrėsi tarp tirtų subproduktų, tačiau reikšmingai priklausė nuo gyvulio rūšies – didžiausios koncentracijos nustatytos galvijų mėginiuose, palyginti su kiaulių ir avių mėginiais ($p < 0,05$).

3.3. Liofilizuotų valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų cheminė ir biologinė sauga

Mikrobiologinių rodiklių analizė pateikta 3.3.1 lentelėje, kurioje nurodyta liofilizuotų valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų mikrobiologinė kokybė. Vertinant tą patį subproduktą ir subproduktų grupę, tarp skirtingų gyvūnų rūšių nustatyti statistiškai reikšmingi bendro aerobinių mikroorganizmų kiekio (TAMC) skirtumai ($p < 0,05$). Tarp kiaulių ir galvijų plaučių mėginių reikšmingų skirtumų nenustatyta, jų TAMC reikšmės abiejose subproduktų grupėse buvo žymiai didesnės nei avių plaučių mėginiuose.

Kepenų mėginiuose abiejose subproduktų grupėse taip pat nustatyti statistiškai reikšmingi skirtumai tarp gyvūnų rūšių ($p < 0,05$). Valgomųjų suproduktų grupėje kiaulių ir avių kepenų TAMC reikšmės buvo didesnės nei galvijų, o trečios kategorijos produktuose didžiausias TAMC kiekis nustatytas avių kepenyse. Inkstų mėginiuose abiejose subproduktų grupėse avių ir galvijų TAMC reikšmės buvo statistiškai reikšmingai didesnės, palyginti su kiaulių mėginiais ($p < 0,05$). Tiek valgomųjų, tiek 3 kategorijos suproduktų grupėse kiaulių širdžių TAMC reikšmės buvo daug didesnės nei avių ir galvijų širdžių ($p < 0,05$), o 3 kategorijos gyvūninių šalutinių produktų mėginiuose skirtumų tarp galvijų ir avių širdžių nenustatyta. Nepriklausomai nuo gyvulio rūšies ar subproduktų grupės, visuose tirtuose subproduktų mėginiuose *Escherichia coli* kiekis buvo mažesnis už aptikimo ribą (< 10 CFU/g). Daugumoje subproduktų mėginių *Enterobacteriaceae* taip pat neviršijo aptikimo ribos, tačiau kepenų mėginiuose jos kartais buvo aptiktos, o jų kiekis svyravo nuo $2,16 \pm 0,12$ iki $2,26 \pm 0,14$ ($\log_{10}$ CFU/g). *Listeria monocytogenes* ir *Salmonella* spp. neaptikta nė viename iš tirtų subproduktų mėginių.

3.3.1 lentelė. *Liofilizuotų valgomųjų subproduktų ir 3 kategorijos šalutinių gyvūninių produktų mikrobiologinė kokybė*

Mėginio kodas	**Bendras aerobinių mikroorganizmų skaičius (TAMC) (log_{10} KSV/g)**	***E. coli*** **(log_{10} CFU/g)**	***Enterobacteriaceae*** **(log_{10} CFU/g)**	***Salmonella*** **spp.**	***Listeria monocytogenes***
Lu-PO-E	4,48 ± 0,15[a]	< 10	< 10	N.D.	N.D.
Lu-OV-E	2,30 ± 0,18[b]	< 10	< 10	N.D.	N.D.
Lu-BO-E	4,60 ± 0,15[a]	< 10	< 10	N.D.	N.D.
Lu-PO-C3	4,62 ± 0,04[a]	< 10	< 10	N.D.	N.D.
Lu-OV-C3	2,51 ± 0,05[b]	< 10	< 10	N.D.	N.D.
Lu-BO-C3	4,65± 0,16[a]	< 10	< 10	N.D.	N.D.
Li-PO-E	3,85± 0,12[a]	< 10	2,16 ± 0,12	N.D.	N.D.
Li-OV-E	3,18 ± 0,04[b]	< 10	< 10	N.D.	N.D.
Li-BO-E	2,30 ± 0,08[c]	< 10	< 10	N.D.	N.D.
Li-PO-C3	3,91 ± 0,15[b]	< 10	2,26 ± 0,14	N.D.	N.D.
Li-OV-C3	4,65 ± 0,07[a]	< 10	< 10	N.D.	N.D.
Li-BO-C3	2,75 ± 0,16[c]	< 10	2,18 ± 0,06	N.D.	N.D.
K-PO-E	3,41 ± 0,06[b]	< 10	< 10	N.D.	N.D.
K-OV-E	3,65 ± 0,19[a]	< 10	< 10	N.D.	N.D.
K-BO-E	3,57 ± 0,06[a]	< 10	< 10	N.D.	N.D.
K-PO-C3	3,49 ± 0,09[b]	< 10	< 10	N.D.	N.D.
K-OV-C3	3,65 ± 0,07[a]	< 10	< 10	N.D.	N.D.
K-BO-C3	3,61 ± 0,15[a]	< 10	< 10	N.D.	N.D.
H-PO-E	4,46 ± 0,08[a]	< 10	< 10	N.D.	N.D.
H-OV-E	3,30 ± 0,12[b]	< 10	< 10	N.D.	N.D.
H-BO-E	3,11 ± 0,04[c]	< 10	< 10	N.D.	N.D.
H-PO-C3	4,45 ± 0,16[a]	< 10	< 10	N.D.	N.D.
H-OV-C3	3,30 ± 0,04[b]	< 10	< 10	N.D.	N.D.
H-BO-C3	3,36 ± 0,06[b]	< 10	< 10	N.D.	N.D.

Skirtingos mažosios raidės ([a–c]) rodo statistiškai reikšmingus skirtumus tarp rūšių tame pačiame subprodukte ir subproduktų grupėje ($p < 0,05$). Lu – plaučiai (angl. *lungs*); Li – kepenys (angl. *liver*); K – inkstai (angl. *kidney*); H – širdys (angl. *heart*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos šalutiniai gyvūniniai produktai pagal Reglamentą (EB) Nr. 1069/2009. N.D. – nenustatyta.

3 kategorijos šalutinių gyvūninių produktų ir valgomųjų subproduktų organinių chlorinių pesticidų (OCP) analizės rezultatai pateikti 3.3.2 lentelėje. Visų analizuotų organinių chlorinių pesticidų kiekiai visuose mėginiuose buvo mažesni už aptikimo ribą (LOD). Aldrino, dieldrino, heptachloro epoksido, chlordanų ar DDT ir jo metabolitų likučių neaptikta.

3.3.2 lentelė. *Organinių chlorinių pesticidų (OCP) likučių vertinimas valgomuosiuose subproduktuose (E) ir 3 kategorijos (C3) šalutiniuose gyvūniniuose produktuose*

Mėginio kodas	**Aldrinas**	**Dieldrinas**	**Heptachloro epoksidas**	**∑ Chlordanų (bendras chlordanų kiekis)**	**∑ DDT (bendras DDT junginių kiekis)**
Lu-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Lu-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Li-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Li-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
Li-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
Li-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Li-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
Li-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
K-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
K-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
K-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
K-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
K-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
K-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
H-PO-E	< LOD	< LOD	< LOD	< LOD	< LOD
H-OV-E	< LOD	< LOD	< LOD	< LOD	< LOD
H-BO-E	< LOD	< LOD	< LOD	< LOD	< LOD
H-PO-C3	< LOD	< LOD	< LOD	< LOD	< LOD
H-OV-C3	< LOD	< LOD	< LOD	< LOD	< LOD
H-BO-C3	< LOD	< LOD	< LOD	< LOD	< LOD

Lu – plaučiai (angl. *lungs*); Li – kepenys (angl. *liver*); K – inkstai (angl. *kidney*); H – širdys (angl. *heart*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos šalutiniai gyvūniniai produktai pagal Reglamentą (EB) Nr. 1069/2009; LOD – aptikimo riba.

Gyvsidabrio (Hg), kadmio (Cd) ir švino (Pb) kiekiai skirtingų gyvūnų valgomuosiuose subproduktuose (E) ir 3 kategorijos gyvūniniuose šalutiniuose produktuose (C3) pateikti 3.3.3 lentelėje. Hg ir Pb kiekiai visuose tirtuose mėginiuose buvo mažesni už aptikimo ribą (LOD). Cd aptikta kepenų ir inkstų mėginiuose, o plaučių ir širdies mėginiuose jo kiekiai išliko žemiau aptikimo ribos (LOD). Didžiausi Cd kiekiai nustatyti inkstuose (0,41–0,58 mg/kg), kur jie buvo reikšmingai didesni nei kepenų mėginiuose (0,06–0,09 mg/kg) ($p < 0,05$). Statistiškai reikšmingų skirtumų tarp valgomųjų subproduktų (E) ir 3 kategorijos šalutinių produktų (C3) nenustatyta ($p > 0,05$).

3.3.3 lentelė. *Sunkiųjų metalų (Hg, Cd ir Pb) kiekiai valgomuosiuose subproduktuose (E) ir 3 kategorijos (C3) šalutiniuose gyvūniniuose produktuose*

Mėginio kodas	Gyvsidabris (mg/kg)	Kadmis (mg/kg)	Švinas (mg/kg)
Lu-PO-E	< LOD	< LOD	< LOD
Lu-OV-E	< LOD	< LOD	< LOD
Lu-BO-E	< LOD	< LOD	< LOD
Lu-PO-C3	< LOD	< LOD	< LOD
Lu-OV-C3	< LOD	< LOD	< LOD
Lu-BO-C3	< LOD	< LOD	< LOD
Li-PO-E	< LOD	0,08 ± 0,01[a]	< LOD
Li-OV-E	< LOD	0,06 ± 0,01[a]	< LOD
Li-BO-E	< LOD	0,08 ± 0,01[a]	< LOD
Li-PO-C3	< LOD	0,07 ± 0,01[a]	< LOD
Li-OV-C3	< LOD	0,09 ± 0,01[a]	< LOD
Li-BO-C3	< LOD	0,08 ± 0,01[a]	< LOD
K-PO-E	< LOD	0,42 ± 0,07[b]	< LOD
K-OV-E	< LOD	0,56 ± 0,04[b]	< LOD
K-BO-E	< LOD	0,48 ± 0,07[b]	< LOD
K-PO-C3	< LOD	0,41 ± 0,04[b]	< LOD
K-OV-C3	< LOD	0,58 ± 0,08[b]	< LOD
K-BO-C3	< LOD	0,45 ± 0,08[b]	< LOD

3.3.3 lentelės tęsinys

Mėginio kodas	Gyvsidabris (mg/kg)	Kadmis (mg/kg)	Švinas (mg/kg)
H-PO-E	< LOD	< LOD	< LOD
H-OV-E	< LOD	< LOD	< LOD
H-BO-E	< LOD	< LOD	< LOD
H-PO-C3	< LOD	< LOD	< LOD
H-OV-C3	< LOD	< LOD	< LOD
H-OV-C3	< LOD	< LOD	< LOD

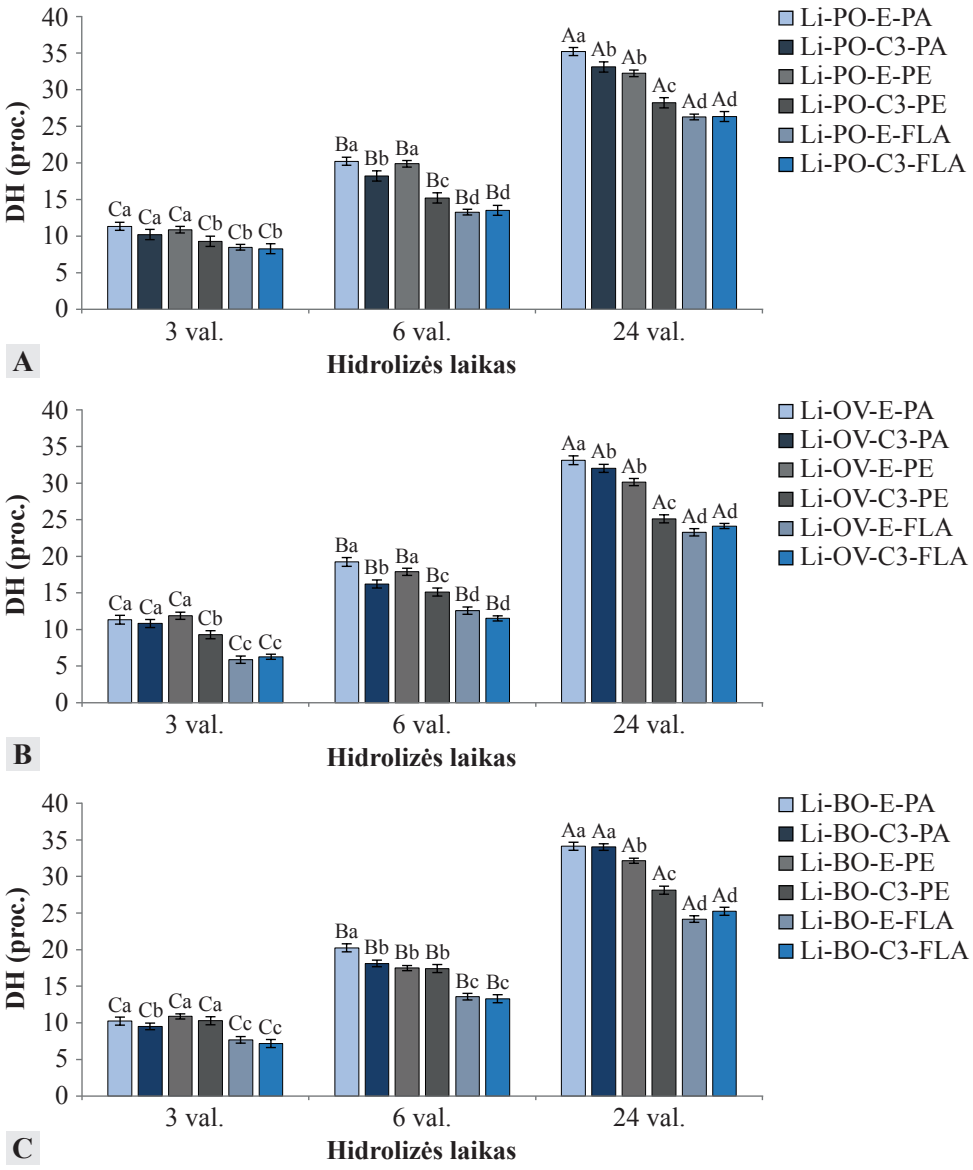
Skirtingos mažosios raidės ($^{a–b}$) nurodo statistiškai reikšmingus skirtumus tarp valgomųjų subproduktų ($p < 0,05$). Lu – plaučiai (angl. *lungs*); Li – kepenys (angl. *liver*); K – inkstai (angl. *kidney*); H – širdys (angl. *heart*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos šalutiniai gyvūniniai produktai pagal Reglamentą (EB) Nr. 1069/2009; LOD – aptikimo riba.

3.4. Fermentinio apdorojimo poveikis kepenų hidrolizės laipsniui

Fermentinio apdorojimo poveikis kepenų mėginių (E ir C3) hidrolizės laipsniui (DH) pateiktas 3.4.1 pav. Kiaulių kepenų baltymų hidrolizės laipsniui (DH) (3.4.1 pav. A) reikšmingą įtaką turėjo apdorojimas fermentais, hidrolizės trukmė ir jų sąveika ($p < 0,001$). DH vertės reikšmingai didėjo ilgėjant hidrolizės trukmei ($p < 0,001$). Didžiausias hidrolizės laipsnis visuose mėginiuose pastebėtas po 24 val. Po 3 val. DH vertės svyravo nuo 8,28 ± 0,21 proc. iki 11,34 ± 0,85 proc. Po 6 val. hidrolizės DH padidėjo iki 13,28 ± 0,24 proc. – 20,23 ± 0,25 proc., o po 24 val. vertės pasiekė 26,28 ± 0,44 proc. – 35,22 ± 0,33 proc.

Avių kepenų hidrolizatų DH (3.4.1 pav. B) statistiškai reikšmingai veikė naudotas fermentas, hidrolizės trukmė ir jų sąveika ($p < 0,001$). DH vertės reikšmingai didėjo ilgėjant hidrolizės trukmei ($p < 0,001$). Po 3 val. DH svyravo nuo 5,88 ± 0,33 proc. iki 12,21 ± 0,52 proc., o po 6 val. padidėjo iki 11,52 ± 0,46 proc.–19,23 ± 0,36 proc. Didžiausios DH vertės užfiksuotos po 24 val. hidrolizės (23,28 ± 0,62 proc.–33,12 ± 0,42 proc.).

Panaši tendencija nustatyta ir galvijų kepenyse (3.4.1 pav. C), kur DH statistiškai reikšmingai priklausė nuo fermentinio apdorojimo, hidrolizės trukmės ir jų sąveikos ($p < 0,001$). Po 3 val. DH vertės svyravo nuo 7,18 ± 0,35 proc. iki 10,88 ± 0,40 proc. Po 6 val. DH padidėjo iki 13,30 ± 0,42 proc.–20,25 ± 0,42 proc., o po 24 val. vertės svyravo nuo 24,18 ± 0,60 proc. iki 34,12 ± 0,52 proc.



3.4.1 pav. *Kepenų hidrolizatų hidrolizės laipsnio (DH) nustatymas naudojant pepsiną ir papainą skirtingais hidrolizės tarpsniais*

(A) Kiaulių kepenys; **(B)** avių kepenys; **(C)** galvijų kepenys. Skirtingos didžiosios raidės (A–C) žymi statistiškai reikšmingus skirtumus tarp skirtingų hidrolizės trukmių to paties subprodukto mėginiuose ($p < 0,001$). Skirtingos mažosios raidės (a–d) esant tam pačiam hidrolizės tarpsniui rodo statistiškai reikšmingus skirtumus tarp fermentinio apdorojimo būdų ($p < 0,001$). Li – kepenys (angl. *liver*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); PA – papainas; PE – pepsinas; FLA – „Flavourzyme“; E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos gyvūniniai šalutiniai produktai pagal Reglamentą (EB) Nr. 1069/2009.

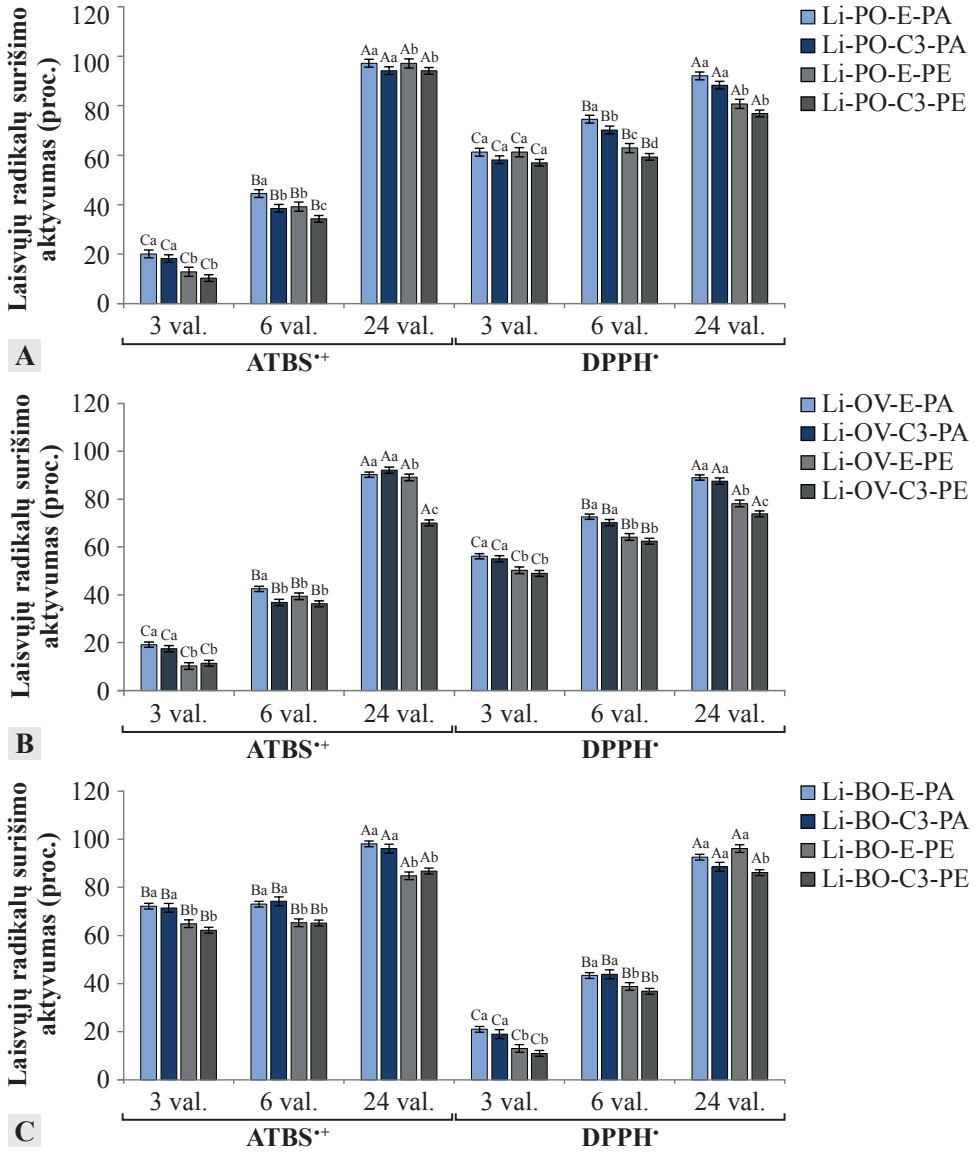
Visuose mėginiuose visais hidrolizės tarpsniais pastebėti reikšmingi skirtumai tarp apdorojimo fermentais ($p < 0,001$). Visų substratų atveju papaino apdorojimo DH vertės buvo didžiausios, o „Flavourzyme“ apdorojimo – reikšmingai mažesnės. Pepsinu apdorotų mėginiai pasižymėjo tarpinėmis DH vertėmis.

3.5. Kepenų hidrolizatų antioksidacinis aktyvumas (ABTS$^{\bullet+}$ ir DPPH$^{\bullet}$ metodais)

Kiaulių, galvijų ir avių kepenų baltymų hidrolizatų, gautų hidrolizuojant skirtingais fermentais 3, 6 ir 24 val., antioksidacinės savybės (ABTS$^{\bullet+}$ ir DPPH$^{\bullet}$ laisvųjų radikalų surišimo aktyvumas) pateiktos 3.5.1 pav.

Kiaulių kepenų hidrolizatų antioksidacinis aktyvumas buvo vertinamas ABTS$^{\bullet+}$ ir DPPH$^{\bullet}$ metodais (3.5.1 pav. A). Nustatyta, kad jis statistiškai reikšmingai priklausė nuo fermento tipo, substrato grupės (E ir C3), hidrolizės trukmės ir jų sąveikos ($p < 0,001$). Hidrolizės trukmė turėjo ryškų poveikį radikalų surišimo aktyvumui, kuris reikšmingai didėjo nuo 3 iki 24 val. ($p < 0,001$). ABTS$^{\bullet+}$ metodu didžiausias aktyvumas nustatytas po 24 val. hidrolizės: 97,20 ± 1,79 proc. valgomųjų subproduktų mėginiuose ir 94,20 ± 1,59 proc. C3 kategorijos mėginiuose naudojant papainą. Panašūs rezultatai gauti ir DPPH$^{\bullet}$ metodu: didžiausios reikšmės po 24 val. siekė 92,07 ± 2,23 proc. (E) ir 88,33 ± 2,01 proc. (C3). Papainu apdoroti mėginiai visais hidrolizės tarpsniais pasižymėjo reikšmingai didesniu antioksidaciniu aktyvumu nei pepsinu apdoroti hidrolizatai ($p < 0,001$). Kepenų (E) hidrolizatai pasižymėjo šiek tiek didesniu aktyvumu nei C3 kategorijos mėginiai, tačiau hidrolizės trukmės poveikis buvo didesnis nei substrato grupės.

Avių kepenų hidrolizatų antioksidacinis aktyvumas taip pat vertintas ABTS$^{\bullet+}$ ir DPPH$^{\bullet}$ metodais (3.5.1 pav. B). Antioksidacinį aktyvumą statistiškai reikšmingai veikė fermento tipas, subprodukto grupė (E ir C3), hidrolizės trukmė ir šių veiksnių tarpusavio sąveika ($p < 0,001$). Hidrolizės trukmė reikšmingai didino radikalų surišimo aktyvumą nuo 3 iki 24 val. ($p < 0,001$). Įvertinus ABTS$^{\bullet+}$ metodu, po 3 val. aktyvumas buvo 19,21 ± 1,26 proc. (E) ir 17,52 ± 0,81 proc. (C3) naudojant papainą bei 10,27 ± 1,57 proc. (E) ir 11,44 ± 1,43 proc. (C3) naudojant pepsiną. Po 6 val. hidrolizės nustatytas antioksidacinio aktyvumo padidėjimas iki 42,50 ± 1,35 proc. (E) ir 36,85 ± 1,87 proc. (C3) kepenų hidrolizatuose, hidrolizuotuose papainu, bei iki 39,41 ± 1,46 proc. (E) ir 36,28 ± 1,01 proc. (C3) mėginiuose, hidrolizuotuose pepsinu. Didžiausias aktyvumas nustatytas po 24 val. – 90,24 ± 2,01 proc. (E) ir 92,12 ± 1,59 proc. (C3) papaino mėginiuose, o pepsino hidrolizatuose – 89,10 ± 1,81 proc. (E) ir 70,10 ± 1,26 proc. (C3).



3.5.1 pav. *Kepenų hidrolizatų, gautų naudojant pepsiną ir papainą, antioksidacinis aktyvumas (ABTS•+ ir DPPH•) skirtingais hidrolizės tarpsniais*

(A) Kiaulių kepenys; **(B)** avių kepenys; **(C)** galvijų kepenys. Reikšmės pateikiamos kaip vidurkis ± standartinis nuokrypis (SD). Skirtingos didžiosios raidės (A–C) žymi statistiškai reikšmingus skirtumus tarp hidrolizės trukmių esant tam pačiam fermentui ir subproduktų grupei, o skirtingos mažosios raidės (a–d) – statistiškai reikšmingus skirtumus tarp fermentų ir kategorijų esant tai pačiai hidrolizės trukmei. Statistinis reikšmingumas nustatytas taikant trijų veiksnių dispersinę analizę (kategorija × fermentas × laikas), po kurios atliktas *Tukey's HSD post hoc* testas ($p < 0,001$). Li – kepenys (angl. *liver*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); PA – papainas; PE – pepsinas; E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos gyvūniniai šalutiniai produktai pagal Reglamentą (EB) Nr. 1069/2009.

Panaši nuo hidrolizės trukmės priklausanti tendencija stebėta ir taikant DPPH$^{\bullet}$ metodą. Po 3 val. aktyvumas siekė 56,15 ± 1,10 proc. (E) ir 55,09 ± 1,67 proc. (C3) naudojant papaino fermentą bei 50,28 ± 1,88 proc. (E) ir 48,98 ± 1,22 proc. (C3) naudojant pepsiną. Po 6 val. aktyvumas padidėjo iki 72,69 ± 1,67 proc. (E) ir 70,22 ± 1,31 proc. (C3) mėginius hidrolizuojant papainu ir iki 64,22 ± 1,82 proc. (E) ir 62,43 ± 1,85 proc. (C3) hidrolizuojant pepsinu. Didžiausias DPPH$^{\bullet}$ aktyvumas nustatytas po 24 val. – 89,06 ± 2,23 proc. (E) ir 87,56 ± 1,14 proc. (C3) hidrolizę atlikus su papaino fermentu.

Galvijų kepenų hidrolizatų antioksidacinis aktyvumas taip pat vertintas ABTS$^{\bullet+}$ ir DPPH$^{\bullet}$ metodais (3.5.1 pav. C). Taikant ABTS$^{\bullet+}$ metodą, aktyvumas jau po 3 val. buvo santykinai didelis ir reikšmingai nesiskyrė nuo 6 val. rezultatų. Statistiškai reikšmingas padidėjimas nustatytas tik po 24 val. hidrolizės ($p < 0,001$), kai antioksidacinis aktyvumas hidrolizuotose kepenyse su papainu siekė 98,10 ± 0,30 proc. (E) ir 96,10 ± 0,30 proc. C3 kategorijos žaliavoje. Panaši tendencija nustatyta ir pepsinu paveiktuose hidrolizatuose – tarp 3 ir 6 val. reikšmingų skirtumų nenustatyta ($p > 0,05$), tačiau po 24 val. fiksuotas reikšmingas padidėjimas ($p < 0,001$).

DPPH$^{\bullet}$ metodu nustatytas ir statistiškai reikšmingas radikalų surišimo aktyvumo padidėjimas po 24 val. hidrolizės ($p < 0,001$). Didžiausios antioksidacinio aktyvumo reikšmės nustatytos kepenų hidrolizatuose, hidrolizuotuose papaino fermentu: 96,12 ± 1,11 proc. (E) ir 86,12 ± 1,36 proc. (C3).

3.6. Kepenų hidrolizatų antibakterinis aktyvumas

Kepenų hidrolizatų antibakterinio aktyvumo slopinant penkias bakterijų padermes – *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 13932, *Salmonella Typhimurium* ATCC 14028 ir *Bacillus cereus* ATCC 11778 – rezultatai po 3, 6 ir 24 val. hidrolizės pateikti 3.6.1 lentelėje.

Stipriausias antibakterinis aktyvumas nustatytas kiaulių kepenų (PO) hidrolizatuose, gautuose naudojant papainą po 24 val. hidrolizės. Mėginys Li-PO-E-PA pasižymėjo stipriu poveikiu slopinant *L. monocytogenes* (22,0 ± 0,2 mm) ir *B. cereus* (19,5 ± 0,1 mm), taip pat vidutiniu poveikiu prieš *S. Typhimurium* (12,5 ± 0,2 mm). Panaši tendencija nustatyta ir Li-PO-C3-PA mėginyje, nors slopinimo zonos buvo šiek tiek mažesnės. Po 24 val. hidrolizės slopinimo zonos reikšmingai padidėjo, palyginti su trumpesnės hidrolizės rezultatais ($p < 0,05$). Kiaulių kepenų hidrolizatai, hidrolizuoti naudojant pepsino fermentą, po 3 ir 6 val. hidrolizės slopinamuoju poveikiu nepasižymėjo. Antibakterinis aktyvumas nustatytas tik po 24 val., kai Li-PO-E-PE slopino *L. monocytogenes* (15,8 ± 0,1 mm) ir *B. cereus* (14,2 ± 0,1 mm), o Li-PO-C3-PE – *L. monocytogenes* (15,0 ± 0,1 mm) ir *B. cereus* (13,0 ± 0,1 mm).

3.6.1 lentelė. *Kepenų hidrolizatų, gautų naudojant pepsiną ir papainą, antibakterinis aktyvumas*

Mėginio kodas	Laikas (val.)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*L. monocytogenes* ATCC 13932	*S. Typhimurium* ATCC 14028	*B. cereus* ATCC 11778
Li-PO-E-PA	3	N.D.	BS	$10,2 \pm 0,1^{b}$	$8,5 \pm 0,2^{b}$	$8,0 \pm 0,1^{c}$
Li-PO-C3-PA		N.D.	BS	$9,8 \pm 0,1^{b}$	$9,2 \pm 0,1^{b}$	$8,2 \pm 0,1^{c}$
Li-PO-E-PA	6	N.D.	BS	$10,7 \pm 0,1^{b}$	$8,6 \pm 0,2^{b}$	$8,5 \pm 0,2^{b}$
Li-PO-C3-PA		N.D.	BS	$9,5 \pm 0,2^{b}$	$8,0 \pm 0,1^{c}$	$10,2 \pm 0,2^{b}$
Li-PO-E-PA	24	N.D.	BS	$22,0 \pm 0,2^{a}$	$12,5 \pm 0,2^{a}$	$19,5 \pm 0,1^{a}$
Li-PO-C3-PA		N.D.	BS	$16,5 \pm 0,2^{a}$	$10,0 \pm 0,1^{a}$	$15,6 \pm 0,2^{a}$
Li-PO-E-PE	3	N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-E-PE	6	N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-PO-E-PE	24	N.D.	BS	$15,8 \pm 0,1$	N.D.	$14,2 \pm 0,1$
Li-PO-C3-PE		N.D.	BS	$15,0 \pm 0,1$	N.D.	$13,0 \pm 0,1$
Li-OV-E-PA	3	N.D.	BS	$10,2 \pm 0,1^{c}$	$10,0 \pm 0,1^{a}$	N.D.
Li-OV-C3-PA		N.D.	BS	$10,0 \pm 0,1^{b}$	$9,2 \pm 0,1^{b}$	N.D.
Li-OV-E-PA	6	N.D.	BS	$11,7 \pm 0,1^{b}$	$10,0 \pm 0,1^{a}$	N.D.
Li-OV-C3-PA		N.D.	BS	$10,0 \pm 0,1^{b}$	$10,0 \pm 0,1^{a}$	N.D.
Li-OV-E-PA	24	N.D.	BS	$17,5 \pm 0,1^{a}$	$10,0 \pm 0,1^{a}$	N.D.
Li-OV-C3-PA		N.D.	BS	$17,0 \pm 0,1^{a}$	$10,0 \pm 0,1^{a}$	N.D.

3.6.1 lentelės tęsinys

Mėginio kodas	Laikas (val.)	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*L. monocytogenes* ATCC 13932	*S. Typhimurium* ATCC 14028	*B. cereus* ATCC 11778
Li-OV-E-PE	3	N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-E-PE	6	N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-OV-E-PE	24	N.D.	BS	N.D.	N.D.	N.D.
Li-OV-C3-PE		N.D.	BS	N.D.	N.D.	N.D.
Li-BO-E-PA	3	N.D.	BS	$9,8 \pm 0,1^{c}$	$10,0 \pm 0,1^{a}$	N.D.
Li-BO-C3-PA		N.D.	BS	$9,0 \pm 0,1^{c}$	$9,2 \pm 0,1^{b}$	N.D.
Li-BO-E-PA	6	N.D.	BS	$11,7 \pm 0,2^{b}$	$10,0 \pm 0,1^{a}$	N.D.
Li-BO-C3-PA		N.D.	BS	$11,2 \pm 0,2^{b}$	$10,0 \pm 0,1^{a}$	N.D.
Li-BO-E-PA	24	N.D.	BS	$17,5 \pm 0,2^{a}$	$10,0 \pm 0,2^{a}$	N.D.
Li-BO-C3-PA		N.D.	BS	$17,8 \pm 0,2^{a}$	$10,0 \pm 0,1^{a}$	N.D.
Li-BO-E-PE	3	N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-E-PE	6	N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-C3-PE		N.D.	N.D.	N.D.	N.D.	N.D.
Li-BO-E-PE	24	N.D.	BS	N.D.	N.D.	N.D.
Li-BO-C3-PE		N.D.	BS	N.D.	N.D.	N.D.

Skirtingos viršutinės raidės ($^{a–c}$) prie to paties mikroorganizmo ir fermento nurodo statistiškai reikšmingus skirtumus tarp hidrolizės trukmių ($p < 0,05$). Li – kepenys (angl. *liver*); PO – kiaulės (angl. *porcine*); OV – avys (angl. *ovine*); BO – galvijai (angl. *bovine*); PA – papainas; PE – pepsinas; E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos gyvūniniai šalutiniai produktai pagal Reglamentą (EB) Nr. 1069/2009. N.D. – slopinimas nenustatytas (angl. *no inhibition was detected*); BS – bakteriostatinis poveikis.

Galvijų kepenų hidrolizatai, gauti 24 val. naudojant papainą, taip pat pasižymėjo antibakteriniu aktyvumu, ypač prieš *L. monocytogenes* (17,5 ± 0,2 mm) ir *S. Typhimurium* (10,0 ± 0,1 mm) – nustatyti reikšmingi skirtumai, palyginti su trumpesnės hidrolizės rezultatais ($p < 0,05$). O galvijų hidrolizatai, gauti naudojant pepsiną, pasižymėjo tik bakteriostatiniu poveikiu bakterijoms *S. aureus*.

Silpniausias antibakterinis aktyvumas nustatytas avių kepenų hidrolizatuose. Papainu apdoroti Li-OV-PA mėginiai pasižymėjo vidutiniu aktyvumu prieš *L. monocytogenes*, didėjančiu ilgėjant hidrolizės trukmei ir po 24 val. siekusiu 17,5 ± 0,1 mm (Li-OV-E-PA) ir 17,0 ± 0,1 mm (Li-OV-C3-PA) ($p < 0,05$). O *S. Typhimurium* slopinimas išliko mažas ir gana pastovus visos hidrolizės metu (apie 9,2–10,0 mm). Mažiausia slopinamoji koncentracija (MIC) prieš *Listeria monocytogenes* kepenų hidrolizatuose buvo 1,25 µg/ml, kas rodo ryškų antibakterinį aktyvumą.

Atsižvelgiant į nustatytas antibakterines ir antioksidacines savybes, tolesniems tyrimams buvo pasirinkti kiaulių kepenų hidrolizatai po 24 val. hidrolizės, kadangi jie pasižymėjo didesniu aktyvumu nei kiti tirti mėginiai.

3.7. Peptidų identifikavimas kiaulių kepenų hidrolizatuose

Po 24 valandų hidrolizės kiaulių kepenų hidrolizatuose identifikuoti peptidai pateikti 3.7.1 lentelėje. Šie peptidai buvo gauti naudojant du skirtingus proteolitinius fermentus, pepsiną (P) ir papainą (PA). Visi peptidai buvo identifikuoti žmonių maistui skirtų kepenų (LI) ir 3 kategorijos ŠGP priskirtų kepenų mėginiuose. Peptidų sekų analizė parodė, kad valgomųjų subproduktų hidrolizatuose identifikuoti peptidai dažniausiai pasižymėjo stipresniais teigiamais tiesinės priklausomybės ryšiais su antioksidaciniu aktyvumu, palyginti su peptidais, identifikuotais 3 kategorijos mėsos šalutinių produktų hidrolizatuose. Kiekvieno hidrolizato antioksidacinis aktyvumas buvo nustatytas DPPH$^{\bullet}$ ir ABTS$^{\bullet+}$ laisvųjų radikalų surišimo metodais, taip pat įvertinti šių rodiklių koreliaciniai ryšiai su LC–MS/MS metodu identifikuotų ir SWATH-MS metodu kiekybiškai įvertintų peptidų pikų plotais.

Stiprūs tiesinės priklausomybės tarp identifikuotų peptidų pikų plotų ir DPPH$^{\bullet}$ antioksidacinio aktyvumo ryšiai nustatyti peptidams: MPVRVVSPDN ($r = 0,896$), VGNLSLGAVQMIGD ($r = 0,884$) ir RSGTPKPSTPTPTP ($r = 0,865$). Taip pat stipriomis teigiamomis tiesinėmis koreliacijomis pasižymėjo peptidai: YNGEEPKETLPFPIID ($r = 0,815$) iš antioksidacinėje apsaugoje dalyvaujančio peroksiredoksino 6 (PRDX6) ir LLRNHPEPT ($r = 0,797$), kilęs iš aldehidooksidazės 1 (AOX1), susijusios su oksidacinėmis reakcijomis.

3.7.1 lentelė. *Kiaulių kepenų hidrolizatuose po 24 val. hidrolizės nustatyti peptidai ir jų tiesinės priklausomybės ryšiai su antioksidaciniu aktyvumu (DPPH$^{\bullet}$ ir ABTS$^{\bullet+}$)*

Peptidų seka	Baltymas	Baltymo biologinė funkcija	Genas	Fermentas	r (DPPH$^{\bullet}$, E/C3)	r (ABTS$^{\bullet+}$ E/C3)	Molekulinė masė (kDa)
YNGEEPKETLPFPIID	Peroksiredoksinas 6	Reaktyviųjų deguonies formų neutralizavimas	PRDX6	P	0,815*/0,675*	0,718*/0,575*	1,862
FDMAAVLQQVL	TPR nukleoproteinas	Branduolio-citoplazmos transporto reguliacija	TPR	P	0,675*/0,221	0,886*/0,664*	1,234
LIRLDST	DENN domeną turintis baltymas 4C	Rab GTPazių aktyvinimas ir vezikulių transportas	DENND4C	P	0,332/N.D.	0,441/N.D.	0,817
AELIFVGMT	Cinko piršto baltymas	DNR prisijungimas ir transkripcijos reguliacija	ZNF280B	P	0,567*/N.D.	0,455/N.D.	0,980
FLAVSDSETRS	Cinko piršto baltymas	DNR prisijungimas ir transkripcijos reguliacija	ZNF280B	P	0,689*/0,568*	0,785*/0,768*	1,211
RSGTPKPSTPTPTP	SPT20 homologas, SAGA komplekso komponentas	Genų ekspresijos reguliacija	SUPT20H	P	0,865*/N.D.	0,765*/N.D.	1,424

3.7.1 lentelės tęsinys

Peptidų seka	Baltymas	Baltymo biologinė funkcija	Genas	Fermentas	r (DPPH•, E/C3)	r ($ABTS^{\bullet+}$ E/C3)	Molekulinė masė (kDa)
IIDAPGHRDF	Eukariotinis transliacijos elongacijos faktorius 1 alfa 1	Aminoacil-tRNR pernaša baltymų sintezės metu	EEF1A1	P	0,234/N.D.	0,123/N.D.	1,140
SDYPPLGRF	Eukariotinis transliacijos elongacijos faktorius 1 alfa 1	Aminoacil-tRNR pernaša baltymų sintezės metu	EEF1A1	P	0,443/N.D.	0,249/N.D.	1,051
VGNLSLGAVQMIGD	Aminooksidazė	Biogeninių aminų oksidacinis skaidymas	LOC100525099	P	0,884*/0,765*	0,887*/0,675*	1,374
LPWGSRGA	Miozinas XVB	Aktino priklausomas viduląstelinis transportas	MYO15B	P	0,768*/0,674*	0,889*/0,765*	0,843
DMHTKPWVR	Betaino–homocisteino S metiltransferazė 1	Homocisteino remetilinimas į metioniną	BHMT	P	0,696*/0,587*	0.567*/0.678*	1,169
LDMHTKPWVR	Betaino–homocisteino S metiltransferazė 1	Homocisteino remetilinimas į metioniną	BHMT	P	0,765*/0,675*	0,567*/0,768*	1,283
QAFDIGTLR	Kadherinas 9	Kalciui priklausoma ląstelių adhezija	CDH9	PA	0,678*	0,675*/N.D.	1,020

3.7.1 lentelės tęsinys

Peptidų seka	Baltymas	Baltymo biologinė funkcija	Genas	Fermentas	r (DPPH•, E/C3)	r (ABTS•+ E/C3)	Molekulinė masė (kDa)
MPRRTH	ATG2A autofagijos baltymas	Autofagosomų formavimosi reguliavimas	ATG2A	PA	0,598*/0,678*	0,686*/0,786*	0,797
LERGEDMLITGGR	Aldehidooksidazė 1	Aldehidų ir ksenobiotikų oksidacija	AOX1	PA	0,765*/N.D.	0,678*/N.D.	1,447
LLRNHPEPT	Aldehidooksidazė 1	Aldehidų ir ksenobiotikų oksidacija	AOX1	PA	0,797*/N.D.	0,885*/N.D.	1,076
LVTASMPGTKT	Unc 80 homologas, NALCN kanalo komplekso subvienetas	Jonų kanalų reguliacija	UNC80	PA	0,495/N.D.	0,396/N.D.	1,105
MPVRVVSPDN	Teniurino transmembraninis baltymas 3	Neuronų jungčių formavimas	TENM3	PA	0,896*/0,881*	0,596*/0,662*	1,113
MQQLRVQLEKMFEA	Unc 13 homologas B	Sinapsinių pūslelių egzocitozės reguliacija	UNC13B	PA	0,556*/0,443	0,695*/0,675*	1,751

Peptidai, kuriems nustatytas bent vienas statistiškai reikšmingas ryšys su antioksidaciniu aktyvumu pagal Pearsono koreliacijos analizę ($p < 0,05$). E – valgomieji subproduktai (angl. *edible offal*); C3 – 3 kategorijos šalutiniai gyvūniniai produktai pagal Reglamentą (EB) Nr. 1069/2009; P – pepsinas; PA – papainas; Li – kiaulių kepenys (angl. *porcine liver*). N.D. – nenustatyta.

Didžiausio stiprumo tiesinės priklausomybės buvo tarp $ABTS^{\bullet+}$ antioksidacinio aktyvumo ir peptidų: LPWGSRGA (r = 0,889), VGNLSLGAVQMIGD (r = 0,887), FDMAAVLQQVL (r = 0,886) ir LLRNHPEPT (r = 0,885), turinčių stiprų laisvųjų radikalų surišimo potencialą. Vidutinio stiprumo teigiamos tiesinės priklausomybės ryšiai nustatyti ir peptidams FLAVSDSETRS, RSGTPKPSTPTPTP, MPRRTH ir MQQLRVQLEKMFEA.

Dauguma identifikuotų peptidų buvo mažos molekulinės masės (< 2 kDa), o tai gali būti siejama su didesniu jų antioksidaciniu aktyvumu ir efektyvesniu laisvųjų radikalų surišimu.

Nors buvo nustatytas hidrolizatų antibakterinis aktyvumas, remiantis naudotomis bioaktyvių peptidų duomenų bazėmis, nė vienam iš LC–MS/MS metodu identifikuotų peptidų antibakterinės savybės nebuvo priskirtos. Todėl koreliacijos analizės apsiribojo ryšiais tarp identifikuotų antioksidacinėmis savybėmis pasižyminčių peptidų ir hidrolizatų antioksidacinio aktyvumo.

Papainu gauti kepenų hidrolizatai iš valgomųjų subproduktų guminukų gamybai parinkti dėl to, kad pasižymėjo didesniu antioksidaciniu aktyvumu, palankesniu peptidų profiliu ir didesniu funkciniu potencialu, palyginti su pepsinu gautais ir iš 3 kategorijos šalutinių produktų pagamintais hidrolizatais.

3.8. Guminukų su kepenų hidrolizatais mikrobiologinė kokybė laikymo metu

Guminukų, paruoštų naudojant kiaulių kepenų hidrolizatus ir agarą kaip stingdančią medžiagą, mikrobiologinė kokybė vertinta 21 dienos laikymo laikotarpiu (3.8.1 lentelė). Nė viename mėginyje per visą laikymo laikotarpį nebuvo aptikta aerobinių mezofilinių bakterijų, išskyrus kontrolinį mėginį (GC), kuriame 21 dieną gyvybingų bakterijų skaičius (TVC) buvo 2,55 $\log_{10}$ CFU/g. Priešingai, visi hidrolizatų turintys mėginiai (GC5LPa3Ag, GC5LPa6Ag ir GC5LPa24Ag) išlaikė TVC vertes žemiau aptikimo ribos (< 1,0 $\log_{10}$ CFU/g) per visą laikymo laikotarpį.

Pelėsių ir mielių nebuvo aptikta nė viename mėginyje jokiu laikymo laikotarpiu, visos vertės išliko žemiau aptikimo ribos (< 1,0 $\log_{10}$ CFU/g).

3.8.1 lentelė. *Guminukų mikrobiologinė kokybė naudojant kepenų hidrolizatus ir gelį formuojančias medžiagas per 21 dienos laikymo laikotarpį*

Mėginių kodai	TVC			Pelėsiai ir mielės		
	1 d.	7 d.	21 d.	1 d.	7 d.	21 d.
	(log_{10} KSV/g)			(log_{10} KSV/g)		
GC	< 1,0	< 1,0	2,55	< 1,0	< 1,0	< 1,0
GC5LPa3Ag	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0
GC5LPa6Ag	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0
GC5Pa24Ag	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0

GC – guminukai; 5,0 – hidrolizato kiekis (g); 3, 6 ir 24 val. – hidrolizės laikas; Pa – papaino hidrolizė; Ag – agaras.

3.9. Guminukų su kepenų hidrolizatais maistinė sudėtis

Guminukų maistinė sudėtis pateikta 3.9.1 lentelėje. Papildymas kiaulių kepenų hidrolizatais turėjo reikšmingą poveikį guminukų maistinei vertei. Baltymų kiekis reikšmingai skyrėsi tarp kontrolinio ir hidrolizatais papildytų mėginių ($p < 0,05$). Mažiausias baltymų kiekis nustatytas kontroliniame mėginyje (GC) ($0,04 \pm 0,01$ g/100 g), o hidrolizatų turinčiuose mėginiuose baltymų kiekis buvo ženkliai didesnis ir svyravo nuo $4,48 \pm 0,02$ iki $4,56 \pm 0,03$ g/100 g.

3.9.1 lentelė. *Guminukų su kepenų hidrolizatais sudėtis (g/100 g)*

Mėginių kodai	Baltymai, g/100 g	Riebalai, g/100 g	Pelenai, g/100 g	Angliavandeniai, g/100 g
GC	$0,04 \pm 0,01^{a}$	$0,03 \pm 0,01^{a}$	$0,16 \pm 0,02^{a}$	$14,57 \pm 0,03^{b}$
GC5LPa3Ag	$4,48 \pm 0,02^{b}$	$0,04 \pm 0,01^{a}$	$0,44 \pm 0,03^{b}$	$19,94 \pm 0,02^{a}$
GC5LPa6Ag	$4,53 \pm 0,0^{b}$	$0,03 \pm 0,01^{a}$	$0,46 \pm 0,03^{b}$	$20,18 \pm 0,02^{a}$
GC5LPa24Ag	$4,56 \pm 0,03^{b}$	$0,04 \pm 0,01^{a}$	$0,48 \pm 0,02^{b}$	$20,42 \pm 0,03^{a}$

$^{a–c}$ vidutinės reikšmės stulpeliuose, pažymėtos skirtingomis raidėmis, statistiškai reikšmingai skiriasi ($p < 0,05$). Duomenys pateikiami kaip vidurkis ($n = 3$) ± standartinis nuokrypis (SN). GC – guminukai; 5,0 – hidrolizatų kiekis (g); 3, 6 ir 24 val. – hidrolizės trukmė; Pa – hidrolizė papainu; Ag – agaras.

Pelenų kiekis taip pat reikšmingai padidėjo įterpus kiaulių kepenų hidrolizatų ($p < 0,05$). GC nustatytas mažiausias pelenų kiekis ($0,16 \pm 0,02$ g/100 g), o kepenų hidrolizatais papildytuose mėginiuose jis svyravo nuo $0,44 \pm 0,03$ iki $0,48 \pm 0,02$ g/100 g.

Angliavandenių kiekis kontroliniame ir hidrolizatų turinčiuose mėginiuose reikšmingai skyrėsi ($p < 0,05$). GC nustatytas mažiausias angliavan-

denių kiekis (14,57 ± 0,03 g/100 g), o visuose hidrolizatų turinčiuose mėginiuose jis buvo reikšmingai didesnis ir svyravo nuo 19,94 ± 0,02 iki 20,42 ± 0,03 g/100 g. Tačiau tarp hidrolizatų turinčių mėginių reikšmingų angliavandenių kiekio skirtumų nenustatyta. Riebalų kiekis tarp tirtų mėginių statistiškai reikšmingai nesiskyrė ($p > 0,05$).

3.10. Guminukų, papildytų kiaulių kepenų hidrolizatais, antioksidacinis aktyvumas (nustatytas $ABTS^{\bullet+}$ ir $DPPH^{\bullet}$ metodais) ir pH

Kiaulių kepenų hidrolizatais praturtintų guminukų antioksidacinės savybės ($ABTS^{\bullet+}$ ir $DPPH^{\bullet}$ radikalų surišimo aktyvumas) bei pH vertės pateiktos 3.10.1 lentelėje. Kontrolinis mėginys (GC) pasižymėjo mažiausia pH verte (3,00 ± 0,02) ir silpniausiu antioksidaciniu aktyvumu, nustatytu tiek $ABTS^{\bullet+}$ (8,52 ± 0,58 μmol/g), tiek $DPPH^{\bullet+}$ (8,62 ± 0,16 proc.) metodais. Papildžius kiaulių kepenų hidrolizatais, reikšmingai padidėjo tiek pH vertė, tiek antioksidacinis aktyvumas ($p < 0,05$). Ilgėjant hidrolizės trukmei, buvo stebimas laipsniškas radikalų surišimo aktyvumo didėjimas. $ABTS^{\bullet+}$ radikalų surišimo aktyvumas padidėjo nuo 24,60 ± 0,93 μmol/g GC5LPa3Ag mėginyje iki 52,67 ± 0,50 μmol/g ir 67,60 ± 0,98 μmol/g atitinkamai GC5LPa6Ag ir GC5LPa24Ag mėginiuose. Panaši tendencija nustatyta ir vertinant $DPPH^{\bullet}$ radikalų surišimo aktyvumą – jis padidėjo nuo 11,93 ± 0,68 proc. GC5LPa3Ag mėginyje iki 33,29 ± 1,07 proc. ir 49,14 ± 1,00 proc. atitinkamai GC5LPa6Ag ir GC5LPa24Ag mėginiuose.

3.10.1 lentelė. *Kiaulių kepenų hidrolizatais papildytų guminukų antioksidacinis aktyvumas, nustatytas $ABTS^{\bullet+}$ (μmol/g) ir $DPPH^{\bullet}$ (proc.) metodais, bei pH vertės*

Mėginių kodai	pH	$ATBS^{\bullet+}$ radikalų surišimo aktyvumas	$DPPH^{\bullet}$ radikalų surišimo aktyvumas
GC	3,00 ± 0,02[b]	8,52±0,58[d]	8,62±0,16[d]
GC5LPa3Ag	3,66 ± 0,02[a]	24,6±0,93[c]	11,93±0,68[c]
GC5LPa6Ag	3,70 ± 0,03[a]	52,67±0,5[b]	33,29±1,07[b]
GC5Pa24Ag	3,65 ± 0,03[a]	67,6±0,98[a]	49,14±1,0[a]

[a–d] vidurkiai stulpeliuose, pažymėti skirtingomis raidėmis, statistiškai reikšmingai skiriasi ($p < 0,05$). Duomenys pateikiami kaip vidurkis (n = 3) ± standartinis nuokrypis (SD). GC – guminukai; 5,0 – hidrolizatų kiekis (g); 3, 6 ir 24 val. – hidrolizės trukmė; Pa – hidrolizė papainu; Ag – agaras.

3.11. Guminukų, papildytų kiaulių kepenų hidrolizatais, antibakterinis aktyvumas

Kiaulių kepenų hidrolizatais, gautais naudojant papainą ir taikant skirtingą hidrolizės trukmę, papildytų guminukų antibakterinis aktyvumas pateiktas 3.11.1 lentelėje. Kontrolinis mėginys (GC) pasižymėjo silpniausiu antibakteriniu aktyvumu slopinant visas tirtas bakterijas – inhibicijos zonų skersmuo svyravo nuo 11,0 ± 0,1 mm esant *S. aureus* subsp. *aureus* ir *L. monocytogenes* iki 16,0 ± 0,1 mm esant *S. enterica* subsp. *enterica* serovar Typhimurium ($p < 0,05$).

3.11.1 lentelė. *Guminukų su kiaulių hidrolizatais antibakterinis aktyvumas*

Mėginių kodai	Slopinimo zonų skersmuo (mm)				
	***E. coli* ATCC 25922**	***S. aureus subsp. aureus* ATCC 25923**	***L. monocytogenes* ATCC 13932**	***S. enterica* subsp. *enterica* serovar Typhimurium ATCC 14028**	***B. cereus* ATCC 11778**
GC	12,0 ± 0,1^{c}	11,0 ± 0,1^{b}	11,0 ± 0,1^{c}	16,0 ± 0,1^{c}	15,0 ± 0,1^{c}
GC5LPa3Ag	16,0 ± 0,1^{b}	11,0 ± 0,1^{b}	21,0 ± 0,1^{b}	16,0 ± 0,1^{c}	15,0 ± 0,1^{c}
GC5LPa6Ag	29,0 ± 0,2^{a}	27,0 ± 0,1^{a}	30,0 ± 0,2^{a}	19,0 ± 0,1^{b}	30,0 ± 0,3^{a}
GC5Pa24Ag	29,0 ± 0,2^{a}	28,0 ± 0,1^{a}	30,5 ± 0,2^{a}	22,0 ± 0,2^{a}	25,0 ± 0,2^{b}

$^{a–c}$ skirtingomis raidėmis pažymėtuose stulpeliuose vidutinės vertės reikšmingai skiriasi ($p \leq 0,05$); duomenys pateikiami kaip vidurkis ($n = 3$) ± standartinis nuokrypis (SN). GC – guminukai; 5,0 – hidrolizatų kiekis (g); 3, 6 ir 24 val. – hidrolizės laikas, Pa – papaino hidrolizė; Ag – agaras.

Papildžius kiaulių kepenų hidrolizatais, reikšmingai padidėjo guminukų antibakterinis aktyvumas. Mėginys GC5LPa3Ag, papildytas po 3 val. fermentinės hidrolizės gautu baltymų hidrolizatu, pasižymėjo reikšmingai stipresniu slopinamuoju poveikiu *E. coli* (16,0 ± 0,1 mm) ir *L. monocytogenes* (21,0 ± 0,1 mm), palyginti su kontroliniu mėginiu ($p < 0,05$).

Ilgėjant hidrolizės trukmei, antibakterinis aktyvumas didėjo. Mėginys GC5LPa6Ag pasižymėjo stipriu slopinamuoju poveikiu visoms tirtoms bakterijoms – inhibicijos zonų skersmuo siekė 29,0 ± 0,2 mm esant *E. coli*, 27,0 ± 0,1 mm esant *S. aureus* subsp. *aureus*, 30,0 ± 0,2 mm esant *L. monocytogenes*, 19,0 ± 0,1 mm esant *S. enterica* subsp. *enterica* serovar Typhimurium ir 30,0 ± 0,3 mm esant *B. cereus* ($p < 0,05$).

GC5Pa24Ag mėginys pasižymėjo ryškiu antibakteriniu aktyvumu – inhibicijos zonų skersmuo siekė 29,0 ± 0,2 mm esant *E. coli*, 28,0 ± 0,1 mm esant *S. aureus* subsp. *aureus*, 30,5 ± 0,2 mm esant *L. monocytogenes* ir 22,0 ± 0,2

mm esant *S. enterica* subsp. *enterica* serovar Typhimurium. Tačiau antibakterinis aktyvumas prieš *B. cereus* (25,0 ± 0,2 mm) buvo mažesnis nei GC5LPa6Ag mėginyje, jis išliko reikšmingai didesnis nei kontrolinio ir GC5LPa3Ag mėginių ($p < 0,05$).

3.12. Guminukų, papildytų kepenų hidrolizatais, fizikinės ir cheminės savybės bei bendras priimtinumas

Guminukų, pagamintų su kiaulių kepenų hidrolizatais, spalvos parametrai, tekstūros savybės ir bendras priimtinumas pateikti 3.12.1 lentelėje. Kiaulių kepenų hidrolizatų įtraukimas reikšmingai paveikė visus spalvos parametrus (L, a, b*, C* ir h) bei tekstūros kietumą ($p < 0,05$), tačiau reikšmingų skirtumų vertinant bendrą priimtinumą nenustatyta ($p > 0,05$).

3.12.1 lentelė. *Guminukų, pagamintų su kiaulių kepenų hidrolizatais, spalvos parametrai, tekstūros savybės ir bendras priimtinumas*

Mėginių kodai	Spalvų koordinatės, NBS					Tekstūros tvirtumas (mJ)	Bendras priimtinumas
	L*	a*	b*	c*	h		
GC	39,83 ± 0,72^{a}	–0,02 ± 0,54^{a}	–3,03 ± 0,47^{d}	3,06 ± 0,49^{c}	262,23 ± 1,74^{a}	3,33 ± 0,06^{c}	7,5 ± 0,5^{a}
GC5LPa3Ag	35,26 ± 0,42^{b}	0,63 ± 0,06^{a}	6,09 ± 0,04^{a}	6,11 ± 0,06^{a}	84,11 ± 0,62^{c}	3,73 ± 0,15^{b}	7,0 ± 0,8^{a}
GC5LPa6Ag	32,77 ± 0,76^{c}	0,43 ± 0,1^{a}	5,01 ± 0,05^{b}	5,03 ± 0,04^{b}	85,33 ± 0,86^{c}	3,63 ± 0,06^{b}	7,0 ± 0,3^{a}
GC5Pa24Ag	36,01 ± 1,49^{b}	0,30 ± 0,02^{a}	3,74 ± 0,12^{c}	3,73 ± 0,14^{c}	92,76 ± 0,57^{b}	4,63 ± 0,15^{a}	7,0 ± 0,5^{a}

$^{a–c}$ skirtingomis raidėmis pažymėtuose stulpeliuose vidutinės vertės statistiškai skiriasi ($p < 0,05$); duomenys pateikiami kaip vidurkis ($n = 3$) ± standartinis nuokrypis (SN). GC – guminukai; 5,0 – hidrolizatų kiekis (g); 3, 6 ir 24 val. – hidrolizės laikas; Pa – papaino hidrolizė; Ag – agaras.

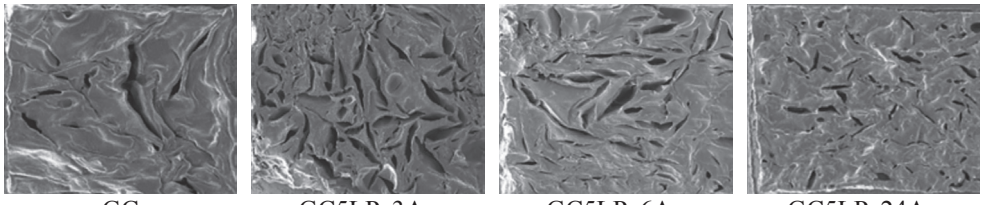
Kontrolinis mėginys (GC) pasižymėjo didžiausiomis šviesumo (L = 39,83 ± 0,72) ir atspalvio kampo (h = 262,23 ± 1,74) reikšmėmis, o hidrolizatų turintys mėginiai pasižymėjo reikšmingai mažesnėmis L reikšmėmis, rodančiomis tamsesnę guminukų spalvą. Papildymas kiaulių kepenų hidrolizatais taip pat lėmė a* ir b* koordinačių pokytį iš neigiamų į teigiamas reikšmes, kas rodo ryškesnių raudonų ir geltonų atspalvių susiformavimą. Didžiausios raudonumo (a* = 0,63 ± 0,06), geltonumo (b* = 6,09 ± 0,04) ir spalvos sodrumo (C* = 6,11 ± 0,06) reikšmės nustatytos GC5LPa3Ag mėginyje, o kontrolinis mėginys pasižymėjo mažiausiomis šių parametrų reikšmėmis.

Guminukų tekstūros kietumas priklausė nuo naudojamų kiaulių kepenų hidrolizatų hidrolizės trukmės. Nors GC5LPa3Ag ir GC5LPa6Ag mėginių kietumas reikšmingai nesiskyrė nuo kontrolinio mėginio, įterpus 24 val. hidrolizės metu gautą hidrolizatą, mėginys GC5LPa24Ag pasižymėjo reikšmingai didesniu kietumu (4,63 ± 0,15 mJ; $p < 0,05$).

Nors nustatyta spalvos ir tekstūros skirtumų, visų mėginių bendro priimtinumo vertinimai buvo panašūs ir svyravo nuo 7,0 ± 0,3 iki 7,5 ± 0,5 balo. Reikšmingų skirtumų tarp mėginių nenustatyta ($p > 0,05$).

3.13. Guminukų mikrostruktūrinė analizė skenuojančiąja elektronine mikroskopija (SEM)

Analizuotų guminukų mikrostruktūriniai skirtumai pateikti 3.13.1 pav. Šių saldainių SEM vaizdai atskleidė akivaizdžius paviršiaus morfologijos skirtumus. Kontrolinis mėginys (GC) pasižymėjo vienalyčiu, nežymiai reljefišku paviršiumi be aiškiai išreikštos porų struktūros. Mėginiai su kepenų hidrolizatais (GC5Pa3Ag, GC5Pa6Ag, GC5Pa24Ag) išsiskyrė didesniu mikroporingumu ir ryškesniu fazių atsiskyrimu.



GC GC5LPa3Ag GC5LPa6Ag GC5LPa24Ag

3.13.1 pav. *Guminukų paviršiaus vaizdai, gauti skenuojančiosios elektroninės mikroskopijos (SEM) metodu, esant 5,0 kV greitinimo įtampai ir 1,00 k× padidinimui; mastelio juosta – 50 µm*

GC – guminukai; 5,0 – hidrolizatų kiekis (g); Pa – papaino hidrolizė; 3, 6 ir 24 val. – hidrolizės laikas, Ag – agaras.

IŠVADOS

1. Veterinarinio tyrimo po skerdimo metu nustatytų gyvulių organų patologinių pokyčių pobūdis neturėjo reikšmingos įtakos cheminei ir biologinei saugai, tačiau turėjo žymią įtaką gyvūninių produktų maistinei sudėčiai ir jų tinkamumui baltymų hidrolizatų gamybai.
   1.1. Didžioji dalis galvijų ir avių organuose nustatytų patologinių pokyčių buvo susiję su virškinimo sistema, o kiaulių organuose – su širdies ir kraujagyslių sistema, vyraujant miokardo hemoragijoms ir endokardito atvejams. Tačiau daugiausia visų gyvulių organuose nustatytų patologijų sudarė kepenų fibroziniai, degeneraciniai ir

hipereminiai pokyčiai, sudarę didžiausią 3 kategorijos šalutinių gyvūninių produktų (ŠGP) dalį.

1.2. Nepriklausomai nuo nustatytų pokyčių, visi analizuoti kiaulių, avių ir galvijų organai atitiko cheminės ir mikrobiologinės saugos ES teisės aktų reikalavimus. Visuose tirtuose mėginiuose gyvsidabrio (Hg), švino (Pb), kadmio (Cd) ir organinių chlorinių pesticidų likučių kiekiai buvo mažesni už Europos Sąjungos reglamentuojamas ribines vertes.

1.3. Nustatyti visų gyvulių organų pokyčiai turėjo įtakos 3 kategorijos ŠGP maistinei sudėčiai. Galima teigti, kad kepenų fibroziniai, degeneraciniai ir hipereminiai pokyčiai mažiausiai paveikė žaliavos maistinę sudėtį – kepenyse nustatytas reikšmingai didesnis baltymų, nepakeičiamųjų aminorūgščių ir polinesočiųjų riebalų rūgščių kiekis, palyginti su kitais pokyčių turinčiais organais ($p < 0,05$).

1.4. Įvertinus tirtų gyvūninių subproduktų biologinę ir cheminę saugą, maistinę sudėtį bei nustatytų patologinių pokyčių pobūdį, nustatyta, kad baltymų hidrolizatų gamybai tinkamiausia žaliava yra kiaulių, avių ir galvijų kepenys, skirtos žmonių maistui, arba dėl fibrozinių, degeneracinių ar hipereminių pokyčių 3 kategorijai priskirti ŠGP. Nustatyta, kad baltymų hidrolizatų gamybai iš širdžių tinkamos tik 3 kategorijos ŠGP priskirtos širdys su hemoraginiais pokyčiais. Inkstai ir plaučiai dėl mažesnio baltymų ir didesnio riebalų kiekio buvo įvertinti kaip mažiausiai fermentinei hidrolizei tinkama žaliava.

2. Fermentinė hidrolizė, atlikta naudojant „Flavourzyme“, pepsiną ir papainą, parodė, kad baltymų hidrolizės laipsnį (DH) reikšmingai veikė fermento tipas ir hidrolizės trukmė ($p < 0,001$). Visuose kepenų mėginiuose DH nuosekliai didėjo nuo 3 iki 24 val., o didžiausios reikšmės nustatytos po 24 val. Iš tirtų fermentų papainas visuose analizuotuose subproduktuose lėmė didžiausias DH reikšmes, o pepsinas pasižymėjo vidutiniu hidrolizės laipsniu. „Flavourzyme“ pasirodė ne itin efektyvus: nustačius mažiausias DH reikšmes visuose tirtuose subproduktuose, tolesniuose eksperimentuose šio fermento atsisakyta.

3. Žmonių maistui skirtų kiaulių kepenų ir 3 kategorijos šalutiniams gyvūniniams produktams priskiriamų kepenų fermentinė hidrolizė papainu ir pepsinu po 24 val. yra perspektyvus metodas siekiant vykdyti biologiškai aktyvių antioksidacinėmis ir antibakterinėmis savybėmis pasižyminčių peptidinių hidrolizatų, skirtų funkcionaliesiems maisto produktams kurti, gavybą.

3.1. Visų tirtų kepenų hidrolizatų antioksidaciniam ir antibakteriniam aktyvumui reikšmingą įtaką turėjo fermento tipas, hidrolizės trukmė ir subproduktų grupė (E ir C3) ($p < 0,001$). Nustatyta, kad hidrolizė papainu lėmė reikšmingai didesnį kepenų peptidų antioksidacinį aktyvumą ir stipresnį antibakterinį poveikį prieš *Listeria monocytogenes* bei *Bacillus cereus*, palyginti su hidrolize pepsinu.

3.2. LC–MS/MS analizė leido identifikuoti potencialiai biologiniu aktyvumu pasižyminčius peptidus, kurių didžiausias antioksidacinis potencialas nustatytas valgomųjų kiaulių kepenų hidrolizatuose. Identifikuotų peptidų pikų plotai pasižymėjo stipriais teigiamais koreliaciniais ryšiais su $DPPH^{\bullet}$ ($r = 0,857–0,896$, $p < 0,01$) ir $ABTS^{\bullet+}$ ($r = 0,885–0,889$, $p < 0,01$) radikalų surišimo aktyvumu.

4. Atrinkti kiaulių kepenų baltymų hidrolizatai po fermentinės hidrolizės su papainu buvo sėkmingai pritaikyti funkcionaliųjų guminukų kūrimui. Hidrolizatų panaudojimas pagerino guminukų antioksidacines ir antibakterines savybes bei padidino jų baltymų kiekį. Didžiausias biologinis aktyvumas nustatytas agaro pagrindu pagamintuose guminukuose su 5 proc. kiaulių kepenų baltymų hidrolizatu, gautu po 24 val. fermentinės hidrolizės su papainu. Nepaisant hidrolizatų sukeltų spalvos ir tekstūros pokyčių, bendras juslinis priimtinumas išliko nepakitęs. Tai patvirtina kiaulių kepenų baltymų hidrolizatų su papainu panaudojimo galimybes funkcionaliųjų produktų gamyboje.

REKOMENDACIJOS

1. Žmonių maistui ir 3 kategorijos šalutiniams gyvūniniams produktams priskirtos kepenys su fibroziniais, degeneraciniais ir hipereminiais pokyčiais, turinčios didelį kiekį baltymų ir pasižyminčios palankiu nepakeičiamųjų aminorūgščių profiliu, yra perspektyvi žaliava peptidinių hidrolizatų gamybai. Siekiant tikslinio 3 kategorijos kepenų panaudojimo funkcionaliųjų ingredientų gavybai, rekomenduojama taikyti išsamesnę jų diferenciaciją, paremtą kompleksiniu patologinių pokyčių pobūdžio ir žaliavos biologinės vertės vertinimu.

2. Kad būtų gauti didžiausiu antioksidaciniu ir antibakteriniu aktyvumu pasižymintys kiaulių kepenų peptidiniai hidrolizatai, rekomenduojama taikyti 24 val. trukmės fermentinę hidrolizę papainu, prieš tai liofilizavus žaliavą. Tokiu būdu gauti peptidiniai hidrolizatai gali būti naudojami funkcionaliųjų maisto produktų arba baltyminių priedų gamyboje.

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1. **Juknienė I**, Zaborskienė G, Jankauskienė A, Kabašinskienė A, Zakarienė G, Bliznikas S. Effect of lyophilization process on nutritional value of meat by-products. Applied Sciences. 2022;12(24):12984. https://doi.org/10.3390/app122412984 Citav. rodiklis: 2.7, bendr. cit. rod.: 5.5, kvartilis: Q2 (2022, InCites JCR SCIE)].
2. **Juknienė I**, Zaborskienė G, Jankauskienė A, Kabašinskienė A, Zakarienė G, Bliznikas S. Effect of enzymatic hydrolysis on functional and biological properties of liver protein hydrolysates. Applied Sciences. 2023;13(24):13142. https://doi.org/10.3390/app132413142 Citav. rodiklis: 2.5, bendr. cit. rod.: 5.5, kvartilis: Q2 (2023, InCites JCR SCIE)].
3. **Juknienė I**, Zaborskienė G, Stankevičienė J. Identification of antioxidant peptides in porcine liver and heart hydrolysates using SWATH-MS analysis. Processes. 2024;12(12):2944. https://doi.org/10.3390/pr12122944 Citav. rodiklis: 3.5, bendr. cit. rod.:3.1, kvartilis: Q2 (2024, InCites JCR SCIE)].
4. **Juknienė I**, Jonnagiri NPKR, Mačionienė I, Zakarienė G, Stankevičienė J, Sinkevičienė I, Radenkovs V, Andrulevičiūtė V, Zaborskienė G. Sustainable formulation of chewing candies using liver hydrolysates with antioxidant and antimicrobial properties. Microorganisms, 2025; 13(8):1882. https://doi.org/10.3390/microorganisms13081882 Citav. rodiklis: 4.2, bendr. cit. rod.:7.7, kvartilis: Q2 (2025, InCites JCR SCIE)].

LIST OF SCIENTIFIC CONFERENCE ABSTRACTS

1. **Juknienė I**, Jankauskienė A, Zaborskienė G. Determination of nutritional value in meat by-products. In: 1st International PhD Student's Conference "Environment – Plant – Animal – Product" (ICDSUPL), Lublin, Poland, April 26, 2022: Vol. 1 (p. 1). Lublin: Publishing House of the University of Life Sciences in Lublin, 2022. [T1e] [M.kr.: A003]
2. **Juknienė I**, Jankauskienė A, Zaborskienė G. The comparison of nutritional value in fresh and lyophilized meat by-products. In: 15th Baltic Conference on Food Science and Technology "Food R&D in the Baltics and Beyond" (FoodBalt 2022), Kaunas, Lithuania, September 26–27, 2022: Abstract book (p. 72). Kaunas: Kaunas University of Technology, 2022. ISBN 9786090218051.
3. **Juknienė I**, Jankauskienė A, Mačionienė I, Zaborskienė G. The impact of enzymatic hydrolysis on antioxidant and antimicrobial properties of ovine meat by-products. In The Vital Nature Sign: 17th International Scientific Conference (Dedicated to the 95th birthday anniversary of Professor Stellan Hjertén): Abstract book, Kaunas, Lithuania, May 18–19, 2023 (p. 51). Kaunas: Vytautas Magnus University, 2023.
4. **Juknienė I**, Zaborskienė G, Zakarienė G, Mačionienė I. Antioxidant and antimicrobial activity of lyophilized meat by-products hydrolysates. In 2nd International PhD Student's Conference Environment – Plant – Animal – Product (ICDSUPL), Lublin, Poland, April 19–20, 2023: Volume 2 (p. 1). Lublin: Publishing House of the University of Life Sciences in Lublin, 2023. ISBN 9788372593900.
5. **Juknienė I**, Stankevičienė J, Stankūnaitė A, Šumskienė M, Zaborskienė G. Analysis of the properties of physiologically active hydrolysates from animal by-products. 4th International PhD Student's Conference Environment – Plant – Animal – Product at the University of Life Sciences in Lublin : 9 April 2025, Lublin, Poland. Lublin : Publishing House of the University of Life Sciences in Lublin, 2025, pp. 1–1.
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7. **Juknienė I**, Zaborskienė G, Falkauskas R. *Assessment of the safety and nutritional value of porcine by-products*. In *5th International PhD Students' Conference: Environment – Plant – Animal – Product: Book of Abstracts* (p. 1). University of Life Sciences in Lublin, Poland, 22–24 April 2026.

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