KAUNAS UNIVERSITY OF TECHNOLOGY

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THE EFFECT OF PLANT ORIGIN INGREDIENTS ON THE QUALITY CHARACTERISTICS AND CHEMICAL PROFILE OF MEAT PRODUCTS

Doctoral dissertation
Natural Sciences, Chemistry (N 003)

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA – Blackcurrant pomace skins after all extractions
AAPH – 2,2‘-azobis(2-amidinopropane) dihydrochloride
$ABTS^{\bullet+}$ – 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
AC – Defatted with supercritical CO_2 rowanberry pomace
ACE – Blackcurrant pomace skins after CO_2 extraction
AUC – Fluorescein (area under the curve) decay curve
BC – Blackcurrant
BC-AE – Blackcurrant seeds after EtOH/water extraction
BC-ASC – Blackcurrant seeds after supercritical fluid CO_2 extraction
BC-RS – Blackcurrant seeds before CO_2 extraction
BHT – Butylated hydroxytoluene
CBD – Cannabidiol
CO_2 – Carbon dioxide
CT – Cycle time in minutes
cvs – Cultivars
DH – Fully defatted hemp seed press-cake flour
DHC – Dihydroxycoumarin
$DPPH^{\bullet}$ – 2,2-Diphenyl-1-picrylhydrazyl
DW – Dry pomace weight
E – EtOH/water extract of defatted with supercritical CO_2 rowanberry pomace
EtOH – Ethanol
GA – Gallic acid
GAE – Gallic acid equivalents
GC–TOF/MS – Gas chromatography coupled to time-of-flight mass spectrometry
GLMM – Generalized linear mixed model
GRAS – Generally Recognized As Safe
H_2O – Water
HCA – Hierarchical cluster analysis
HOE – Combined extract of defatted residues consecutively isolated with EtOH and H_2O
HRMS – High-resolution mass spectrometry
HS-SPME – Headspace solid phase microextraction
HS-SPME-TOF-MS – Headspace solid-phase microextraction combined with time-of-flight mass spectrometry
LC–MS – Liquid chromatography–mass spectrometry
LLE – Liquid-liquid extraction
MAE – Microwave-assisted extraction
MAP – Modified atmosphere packaging
MDA – Malondialdehyde
N_2 – Nitrogen
OPLS-DA – Orthogonal projections to latent structures discriminant analysis
ORAC – Oxygen radical absorbance capacity

PCA – Principal Component Analysis
PBS – Phosphate-buffered saline
PLE – Pressurized liquid extraction
PUFA – Polyunsaturated fatty acids
QC – Quality control GLMM
QUENCHER – Quick, easy, new, cheap and reproducible
R – Extraction residue of rowanberry pomace
RS – Raw blackcurrant pomace skins
RB – Rowanberry
RH – Dried hempseed press-cake flour
RHSG – mixture of dried pressed hempseed cake and sweet grass extract
RI – Retention indices
RNS – Reactive nitrogen species
ROS – Reactive oxygen species
RSC – Radical scavenging capacity
RTE – Ready-to-eat food
$scCO_2$ – Supercritical CO_2 extraction
SD – Standard deviation
SE – Soxhlet extraction
SET – Single electron transfer
SFE – Supercritical fluid extraction
SFE-CO_2 – Supercritical fluid extraction with carbon dioxide
SG – Sweet grass extract
SLE – Solid-liquid extraction
TBARS – Thiobarbituric acid reactive substances
TBHQ – Tertiary butylhydroquinone
TE – Trolox equivalents
THC – Tetrahydrocannabinol
TOF/MS – Time-of-flight mass spectrometry
TPC – Total phenolic content
UAE – Ultrasound assisted extraction
UHPLC – Ultra-high-pressure liquid chromatography
UHPLC-HRMS – Ultra-high-performance liquid chromatography–high resolution mass spectrometry
WHC – Water-holding capacity
WHO – World Health Organization

INTRODUCTION

Since the beginning of human existence, meat has been used for food. Meat and meat products constitute an important source of high biological value proteins, minerals, especially bioavailable iron, vitamins and other essential elements (Lorenzo and Pateiro[1]). It plays a fundamentally important role in the human diet. Global meat consumption has been steadily increasing. According to OECD-FAO Agricultural Outlook 2022[2], world meat production increased by 5% in 2021, led by the 34% increase in pork production, which constitutes the fraction of meat in the world. According to projections, the demand for animal-derived foods would rise 14% globally by 2030 compared to the average of 2018-2020. Several studies have shown meatballs and meat patties to be very popular products in Europe; however such products are quite sensitive to oxidation compared with other meat products. For meat factories, a major challenge is to provide products with a pleasant flavour, colour and freshness throughout the storage period. As the meat production is also related with the environmental impact, there is a clear tendency of developing meat products by partial or full substitution of meat by plant origin-based and non-conventional ingredients (Rocchetti *et al*.[3]). Valorization of food by-products and wastes for this purpose represents a significant step towards environmental balance.

Meat is a highly complicated system consisting of numerous substances. Therefore, different chemical reactions occur during storage and processing, and some of them may negatively affect its quality and safety. Meat, especially minced meat products, is very sensitive to microbiological and oxidation processes causing a variety of quality defects. Oxidation results in discoloration, off-flavours, formation of toxic and health related compounds and an increase in their amount, as well as a shortening shelf-life. Those changes can be controlled via physical methods or by using chemical additives, *e.g.* tertiary butylhydroquinone (TBHQ). Due to the increasing consumer preferences towards 'naturalness' of foods, the application of natural additives instead of synthetic ones presents an attractive alternative. Therefore, there is a rapid growth in the demand for natural bioactive compounds to be used in meat and meat products.

During the processing of horticultural and agricultural crops, together with the main products in many cases, large amounts of by-products are generated (30–50% of dry weight). According to the European Union report, it ranges from 12–41% (Caldeira[4]). These side-streams (skins, seeds, press-cakes, etc.) may contain a significant amount of substances beneficial to health, *e.g.* polyphenols, carotenoids and other nutrients which could be used in animal origin foodstuffs to give added value and increase their quality. Valorization of by-products and the recovery of extracts rich in antioxidants has been gaining popularity. It is known that they contain a high amount of bioactives. The recovery of bioactive compounds from berry pomace is desired as they can be applied in foods, nutraceuticals, pharmaceuticals, and cosmetic products. It has been stated that plant-based by-products may be richer in bioactives than the initial product (Galanakis[5]). By adding a herbal material rich in bioactive compounds, it is possible to significantly slow

down the growth of microorganisms, as well as the oxidation of fatty acids and cholesterol in meat products, which also increases the shelf-life of the products. Research on the functionality and the use of plant-based ingredients is relevant and actual worldwide. Still, only a relatively small part of the potential plant-based materials has been comprehensively studied as meat product ingredients so far. Therefore, it is prominently important and challenging for scientists to find out promising by-products to be used in meat products, and thus increase their quality characteristics, while also prolonging the shelf-life and health-related benefits.

Numerous plant materials, and particularly small fruits (commonly called berries), such as blackcurrants, blueberries, blackberries, cranberries, grapes and others are a good source of natural antioxidants and health-promoting bioactive components, which may also help to stabilize the quality of meat products, as well as reduce the impact of (per)oxidation in meat and extend its shelf-life. In addition, processing of fruits and berries generates large amounts of by-products (called *pomace*) consisting of seeds, skin, and pulp residues. However, nowadays, large amounts of pomace are still discarded as a waste, thus causing the loss of valuable bioactive phytochemicals. Extracts of berries and other plant ingredients are rich in various compounds, *e.g.* polyphenols which are beneficial to health, and which are therefore suitable for using in meat and meat products as the consumers are demanding for healthy and natural food products containing naturally derived antioxidants. Moreover, some 'forgotten' crops, like industrial hemp (*Cannabis sativa* L.) have gained an interest among researchers.

Environmental issues are other significant concerns in the development of effective tools and technologies for the valorization of agri-food waste/by-products generated during food processing. From this perspective, the so-called green extraction processes or methods are given preference in developing novel technologies in various industries including the food industry. Many studies use green extraction methods to recover bioactive compounds from fruit and vegetable processing waste/by-products. Green chemistry-based extraction techniques are preferred over other extraction techniques due to various reasons. For example, green extraction techniques are free from the use of toxic organic solvents, which results in less utilization of energy and other resources. Consequently, the development of multifaceted novel extraction techniques, including supercritical CO_2 and pressurized liquid extraction methods to recover bioactive constituents, could be regarded as a highly promising tendency in the valorization of agri-food waste/by-products. In order to accomplish better outcomes, these extraction techniques could be used in combination with a number of mathematical optimization methods.

Metabolomics is a relatively recent approach in the studies of small molecules (metabolites) as the unique chemical fingerprints, involving the identification and quantification of all the small metabolites. Untargeted metabolomics allows identifying unknown compounds and the biomarkers of spoilage – several lipids and proteins-related oxidation products (Rocchetti *et al.*[6]), which has been receiving an ever increasing interest in the recent years in various food matrices.

This study investigates the possibilities of using various plant ingredients in meat products with the objective to evaluate their effect on the general quality characteristics and the formation of products of chemical reactions.

The aim of the research is to evaluate the possibilities of using selected innovative plant origin ingredients in meat products by assessing their effects on various quality parameters and the formation of products of chemical reactions.

The following objectives are raised to achieve the aim:

(1) To select and characterize innovative and promising plant origin ingredients to be used in meat products and establish the sensorically acceptable dosages of those selected plant ingredients in cooked meat products;
(2) To evaluate the effect of plant origin ingredients on the composition and quality characteristics of cooked meat products;
(3) To evaluate the effect of plant origin ingredients on the meat quality characteristics during storage;
(4) To evaluate the effects of plant ingredients on the formation of products of chemical reactions in meat by using the untargeted metabolomics approach.

Practical significance of the thesis

The results obtained may provide valuable information on the possibilities of improving the overall quality of meat products, which may be also highly attractive practically for meat factories. The extracts obtained via different extraction methods were used in meat products, and therefore they may be considered as a promising way of increasing the oxidative stability and improving the quality of meat products. The results of the thesis can be expected to be useful for quality development of processed meat in the future as the used plant-based ingredients are cheap, easily available, and useful in enriching meat products with health-beneficial phytochemicals.

Scientific novelty

(1) The concept of biorefining blackcurrant (*Ribes nigrum* L.) pomace solid fractions by using green solvents and extraction technologies has been tested for the first time in meat products;
(2) Rowanberry (*Sorbus aucuparia* L.) pomace and sweet grass (*Hierochloe odorata* (L.) P. Beauv.) extract as promising natural antioxidants has not previously tested in meat products;
(3) Untargeted metabolomics as a new approach for assessing quality changes and oxidation processes in meat products has been used.

Publication of the results

Results of the research have been presented in 3 publications and reported at 7 international conferences and 3 national conferences.

Structure and content of the dissertation

The dissertation is written in English. It contains a list of tables and figures, list of abbreviations, an introduction with the review of the applicable literature, materials and methods, results and discussion, conclusions, a list of references (230 in total), and a list of publications on the topic of dissertation. The final dissertation contains 124 pages, including 17 tables and 36 figures.

1. LITERATURE REVIEW

1.1. Trends in the Formulation of Meat Products

Global consumption of meat and meat products has been continuously increasing (Kotecka-Majchrzak *et al.*[7]), and the demand for animal-derived foods is expected to rise 14% globally by 2030 in comparison to the average of 2018-2020, which is why the meat industry is denoted by outstanding importance among food industries. Although, today, consumer preferences have been changing greatly. Natural food ingredients are desired, which, in turn, makes it necessary to use more natural ingredients and additives. There is an emergent tendency in the consumption and manufacture of meat products and semi-finished products, which is because of the modern lifestyle of the customers and their demand for *ready-to-eat* (RTE) foodstuffs. Regardless of the huge range of food products, the selection of food products with functional properties is still somewhat limited, whereas unbalanced nutrition is significant in today's society. Reduction in the content of nitrates, cholesterol, fat and sodium chloride on the one hand, and the improvement of the fatty acid profile on the other hand by adding bioactive compounds are the key directions in the creation of fortified meat products (Gabdukaeva *et al.*[8]). Research is on the go to develop novel meat products with the anticipated properties, whereas their beneficial effects on health are being studied in parallel (Shan *et al.*[9]; Perez-Montes *et al.*[10]). Meat products with a balanced composition of necessary ingredients can increase the nutritional value and be beneficial for people with special nutritional requirements (Gabdukaeva *et al.*[8]). It leads to a change in the consumer demand and an increased international competition, which forces the meat industry to adopt novel processing methods and novel ingredients. This is an important approach in the context of the long-term evolution of products and processes in the meat industry (Verbeke *et al.*[11]).

The use of plant ingredients with antioxidative and antimicrobial properties has a great significance in food preservation and for health-related benefits. Another trend in developing meat products is related to the use of cheaper plant-origin protein substitutes in their formula (Sha *et al.*[12]). According to this, various proteinaceous plant origin ingredients have been tested in meat products, including soya, pea, mung bean, rice, and lupin as the most widely used options (Danowska-Oziewicz *et al.*[13]; Rocchetti *et al.*[3]; Sha *et al.*[12]; Zhang *et al.*[14]).

Different fruit extracts rich in phenolic compounds are also added to muscle foods to retard oxidation and discolouration; these extracts include, among others, pomegranate juice phenolics (Vaithiyanathan *et al.*[15]), white grape extract (Jongberg *et al.*[16]) and extracts from strawberry, dog rose, hawthorn, arbutus berry, and blackberry (Ganhão *et al.*[17]), cranberry and black chokeberry pomace (Tamkutė *et al.*[18]) or rowanberry pomace extracts (Sarv *et al.*[19]) or even cherry and blackcurrant leaf extracts (Nowak *et al.*[20]).

Current trends focus on the use of natural plant extracts in meat products to improve and control their quality so that the consumers could have healthier meat

products. Consumers prefer natural alternatives instead of synthetic antioxidants, and this has resulted in the development of 'clean label' products. Moreover, the consumers' preference for natural food additives and ingredients, which is a concern regarding the safety of synthetic preservatives, has prompted the food industry to look for natural alternatives. Due to potential negative health effects (*e.g.*, toxicological and nutritional issues) of synthetic antioxidant compounds at high concentrations, the application of natural plant-based antioxidants has gained significant attention.

1.2. The Importance and Effect of Plant Origin Ingredients on the Quality of Meat Products

Excessive consumption of meat is not recommended, because it contains high amounts of cholesterol, saturated fats, phosphates, sodium and polyunsaturated fatty acids (Owusu-Ansah *et al*.[21]). Also, attitudes towards meat and its impact on human health have recently become negative (Zajac *et al*.[22]), whereas consumer preferences and growing competition are conducive to innovation. The main disadvantage of meat and meat products is the lack of fibre and the content of saturated fats; hence the need arose to improve the nutritional value of the meat as a whole, which can be done by using non-animal ingredients (Kausar *et al*.[23]).

High consumption of red and processed meat can lead to a higher risk of developing type 2 diabetes and certain types of cancer (Pihlanto *et al*.[24]). In addition, meat contains high cholesterol, fat and saturated fatty acids (40–50%), which, in turn, can lead to obesity and heart disease (Zajac *et al*.[22]). WHO's *International Agency for Research on Cancer* (IARC[25]) has suggested to decrease the consumption processed meat products. Therefore, it is necessary to enhance the overall nutrition quality of meat. To achieve this, non-meat components that can be added to meat to improve its nutritional value and functional qualities can be used. In order to produce healthier meat products, meat factories have started to use functional ingredients in their products (Longato *et al*.[26]). In this light, product developers have been focusing on working out healthier meat products by reducing salt, nitrites, cholesterol, saturated fat content and incorporating various functional ingredients (Dominguez *et al*.[27]; Dominguez *et al*.[28]; Schmiele *et al*.[29]). Among functional ingredients, the importance of fibres has been growing (Zinina *et al*.[30]). Vegetables and fruits are excellent sources of fibres. Fibres from fruits can be obtained via the production of juices as by-products and be successfully used in the formulation of meat products. Fibres are present in fruit skins and are used as effective ingredients due to their excellent technological properties, *e.g.* water-holding capacity, which lowers the cooking loss. Besides all of that, fibres are denoted by numerous healthy effects: a lower risk for developing coronary heart disease, hypertension, diabetes, obesity, and certain gastrointestinal diseases (Anderson *et al*.[31]; Gustafson and Rose[32]). Therefore, natural plant-based ingredients rich in dietary fibre and with a high antioxidant capacity are promising ingredients for the meat industry with an expectation to be used in meat products (Cunha *et al*.[33]; Lorenzo *et al*.[34]; Lunn and Buttriss[35]).

For the consumers, it is increasingly important for the product to be 'clean and safe'; therefore meat factories must reduce the use of synthetic additives. Nitrites, phosphates and glutamate can be replaced with natural ingredients. This has led to an increase in the use of plant extracts, as well as plant-based ingredients, which could allow to produce meat products with better nutritional and sensory properties, and a longer shelf-life (**Fig 1.1.**).

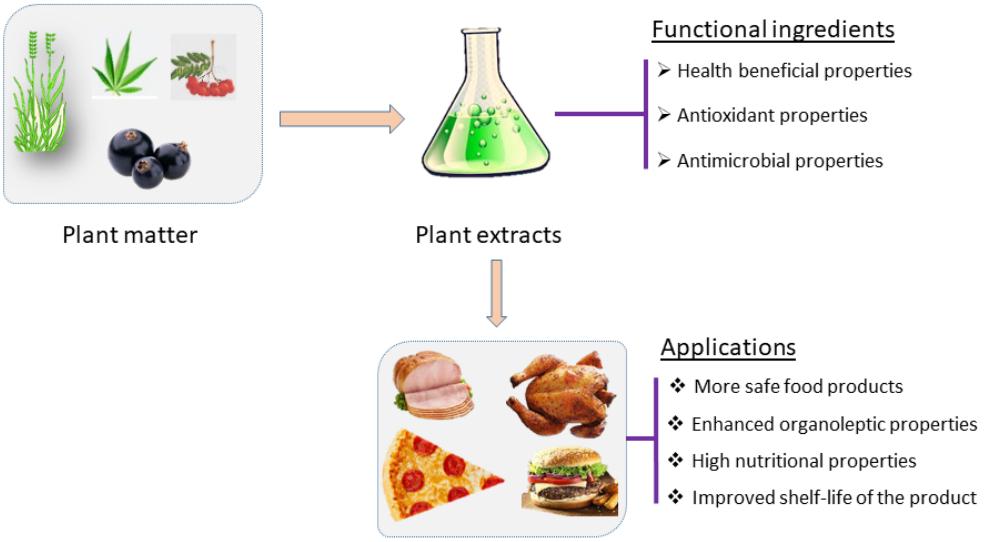

Fig. 1.1. Application of plant extracts in meat products to improve their quality (the figure is composed based on Nikmaram *et al.*[36])

As meat products are sensitive to oxidation processes, different plant-based ingredients, such as extracts from fruits, berries, vegetables and herbs, are used to avoid the deteriorative impact of *reactive oxygen species* (ROS). **Tbl. 1.1.** shows the effect of the incorporation of plant origin ingredients on the quality of various meat products.

The current demand for food proteins has been increasing as the world population is predicted to rise to 9 billion by 2050, and as the availability of animal proteins is increasingly limited, plant substitutes need to be found and introduced (Shen *et al.*[37]). The growth of the world population is generating demand for new and highly nutritious products, in particular products combining both animal and vegetable proteins (Kristensen *et al.*[38]). Findings have shown that the addition of non-meat ingredients not only improves the quality of meat products, but it also lowers their price and enhances the consumers' health.

Table 1.1 Effect of some plant origin ingredients on the quality of various meat products (the table is modified by Biswas *et al*.[39])

Plant ingredients	**Meat products**	**Effect on quality**	**Reference**
Peel powder of dragon fruit	Chicken nuggets	1. Cooking yield and emulsion stability improvement 2. Reduction in hardness, chewiness and gumminess levels 3. Inhibited lipid peroxidation, odour scores and microbial load	Madane *et al.*[40]
Pomegranate peel	Chicken burger	1. Reduced the acid, peroxide and TBA values of their fats 2. Delayed the growth of yeasts and moulds and psychrophilic bacteria 3. Improved the appearance, taste, texture and odour	Aly[41]
Lotus rhizome	Cooked sausage	1. Improved red and darker colour 2. Decreased cooking loss 3. Better chewiness and springiness 4. Enhanced emulsion stability and viscosity 5. Reduced juiciness and colour scores	Ham *et al.*[42]
Sugarcane fibre	Chicken sausage	1. Improved the cooking yield 2. Retarded lipid oxidation 3. Improved the springiness and hardness	Fang *et al.*[43]
Ethanol extract obtained from tomato powder	Pork patties	1. Enhanced the redness value 2. Retarded the bacterial counts 3. Inhibited lipid oxidation	Kim and Chin[44]
Microparticles of plum pulp and peel	Chicken patties	1. Improved redness and springiness 2. Enhanced the antioxidant activity 3. Enhanced textural parameters	Basanta *et al.*[45]
Bamboo shoot extract	Pork nuggets	1. Delayed lipid peroxidation and quality deterioration 2. Improved the microbiological attributes 3. Enhanced the flavour	Thomas *et al.*[46]

1.3. Ingredients Used in the Formulation of Meat Products

1.3.1. Blackcurrant (*Ribes nigrum* L.) pomace ingredients

Blackcurrants (BC) are a species of currant belonging to the family *Grossulariaceae*, which is a species native to central and northern Europe. They are grown for its edible dark-purple, almost black berries, each containing many seeds.

Approximately 15–20% of blackcurrants are consumed as fresh, the rest is used to make jams, syrups and even to make alcoholic beverages. Over 80% of their harvests are processed into numerous products, mainly pressing juice. It generates large amounts of pomace (by-product), containing seeds and skins, but discarded as a waste. Although, an additional value could be given through the extraction of phenolic compounds from these by-products.

The growing area of blackcurrants in Estonia in 2022 was 491 ha (11%) among all grown fruits and berries (Statistics Estonia; PM0281[47]). These fruits are also well-known worldwide with the production of 153 028 metric tons (t) (International Blackcurrant Association[48]).

Blackcurrants are found to possess antioxidant and anti-inflammatory properties due to the high content of phenolic acids, vitamin C, and other flavonoids (Tabart *et al*.[49]; Xu *et al*.[50,51]). Antioxidant, cardioprotective, antiviral, antibacterial, and anticancer effects are all exhibited by flavonoids. Consequently, they should be incorporated into a healthy diet, and also they are great ingredients for many functional food products (Barreca *et al*.[52]). The value of blackcurrant flavonoids has also been approved by the food and pharmaceutical industries due to their antimicrobial capacity (de Araújo *et al*.[53]). Polyphenols are also present in seeds and leaves.

Blackcurrants contain an average of 82% of water, 1.1–1.4 g/100g protein, fat 0.41–1.4 g/100 g, and the carbohydrate content is between 9.7–15.4 g/100 g (Kikas and Libek[54]; NutriData[55]). The main sugars in blackcurrants are fructose, glucose and sucrose (Tian *et al*.[56]). The average chemical composition of BC ingredients depends on the cultivar of the berries, the degree of maturity, the growing region and growing conditions (Kaldmäe *et al*.[57]; Kikas *et al*.[58]). Due to the high biological value of the blackcurrant, as well as the rich and diverse chemical composition, the berries are used both in medicine and in the food industry. The fruits contain 150–300 mg of vitamin C, 8–12% sugar, 3–4% acid, and 1.1% pectin (Petrova *et al*.[59]).

The main anthocyanins (250 mg/100 g fresh fruit) (Bishayee *et al*.[60]), known as water-soluble pigments, found in pomace are delphinidin-3-*O*-glucoside, delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside (**Fig. 1.2.**).

Määttä *et al*.[61] found in their research that, compared to other currants (red, green, and white), BC had the highest content of anthocyanin aglycons and flavonol glycosides, which also depended on the processing and storage method of the berries. It has been found out that anthocyanins in BCs deliver the best antioxidant activity compared with other fruits (Jia *et al*.[62]).

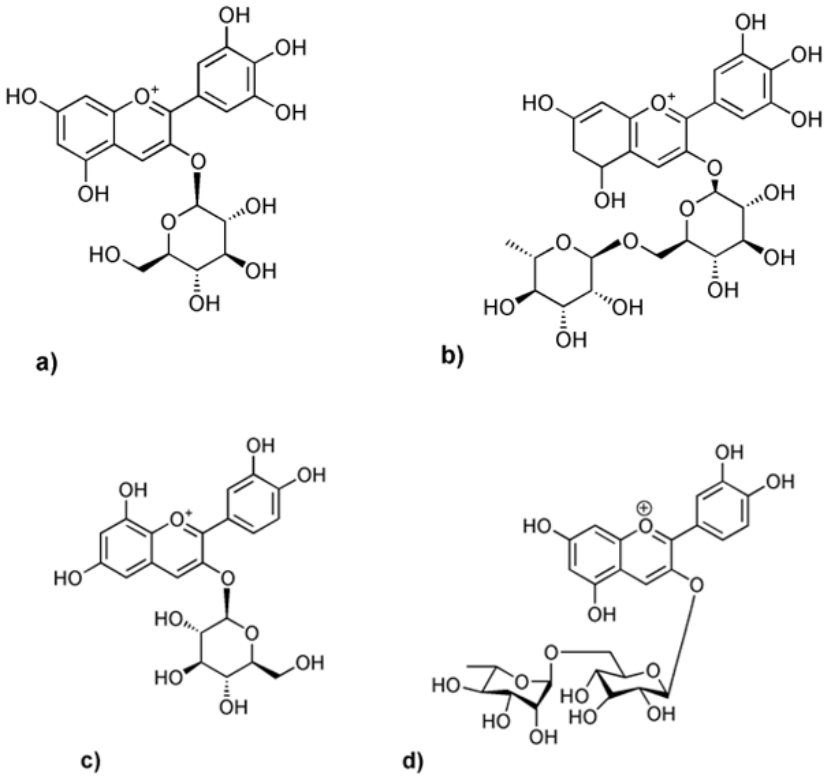

Fig. 1.2. Anthocyanins a) delphinidin-3-*O*-glucoside; b) delphinidin-3-*O*-rutinoside; c) cyanidin-3-*O*-glucoside, and d) cyanidin-3-*O*-rutinoside

In addition, the blackcurrant exhibits the highest *in vitro* antioxidant activity, depending on the cultivar and composition, compared to other berries (Borges *et al*.[63]). On top of that, its commercial by-product, blackcurrant press-cake, has also demonstrated antioxidant activity (dos Santos Lima *et al*.[64]). Therefore, the addition of different blackcurrant pomace ingredients may affect the overall meat quality and have many health benefits including anticarcinogenic properties (Wu *et al*.[65]).

Blackcurrant fruits have a high content of organic acids, which gives them a sour taste. Ascorbic acid is the most abundant water-soluble antioxidant in plant tissues, which humans cannot synthesise. The content of ascorbic acid as a powerful antioxidant and free radical scavenger (RSC) in blackcurrants ranges from 70 to 280 mg/100 g of fresh fruit (Gopalan *et al*.[66]). Jakobek *et al*.[67] compared different berries and found that BC berries were third best in DPPH$^{\bullet}$ radical scavenging properties after chokeberry and bilberry. BC is also rich in phenolic compounds, mainly phenolics and flavonoids (Vagiri[68]). Phenolics have antioxidative, antimicrobial, anti-inflammatory and sensory properties in food (Pisoschi *et al*.[69]). The main phenolic compounds in berry fruits are presented in **Fig. 1.3.**

Additionally, extracts from blackcurrant leaves as natural antimicrobial agents have been tested by Nowak *et al*.[20] in meat products.

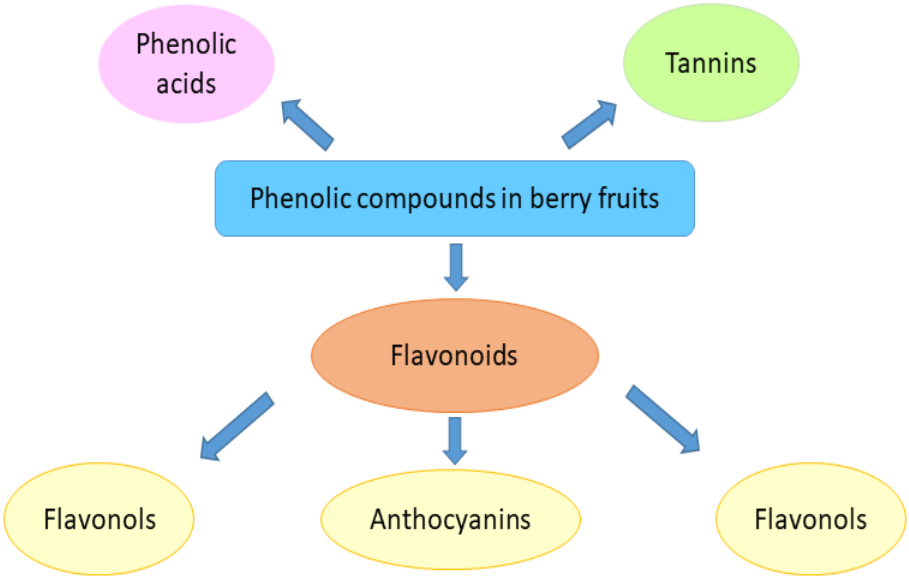

Fig. 1.3. Phenolic compounds in berry fruits (the figure is composed based on Paredes-López *et al.*[70])

Recent studies have indicated that blackcurrant berries have antioxidant and antimicrobial properties both *in vitro* and in the product (Anton *et al.*[71]; Raudsepp *et al.*[72]). Anton *et al.*[71] added different plant powders, including blackcurrant, into raw and cooked minced pork. In raw minced pork, rhubarb roots with blackcurrant berries (1%) significantly inhibited lipid oxidation.

In another study by Jia *et al.*[62], BC extract at 5, 10, or 20 g/kg was added to pork patties under chilled conditions. TBARS value decreased by 91.7% with 20 g/kg dosage. The patties also had higher redness (*a**) values.

Sodium nitrite ($NaNO_2$) is a multipurpose ingredient, which is frequently used in the meat industry. This substance has a potential to cause cancer; therefore, its use should be restricted. Hence, Wójciak *et al.*[73] investigated the effect of enriching canned meat with blackcurrant leaf extract and reducing the sodium nitrate (III) amount added from 100 to 50 mg/kg. On the grounds of the results, it was confirmed that blackcurrant leaf extract might be used to fortify canned meat. It comprises substantial amounts of derivatives of flavonoids and phenolic acids. In addition, it was shown that adding the maximum dose of the three different blackcurrant leaf extract test doses (50, 100, and 150 mg/kg) preserved the antioxidant characteristics of canned meat for 180 days when it was stored at 4 °C. This variant was characterized at the end of the storage period by antiradical activity against $ABTS^{\bullet+}$, and it showed the strongest reducing capacity. Throughout the entire storage period, the addition of 150 mg/kg of blackcurrant leaf extract reduced the oxidative transformations of lipid in meat products, which resulted in reaching a level of TBARS that was nearly two times lower than in the control sample. Furthermore,

these meat products were also characterized by good microbiological quality, while the stability of colour parameters did not involve *N*-nitrosamines.

1.3.2. Sweet grass (*Hierochloe odorata* (L.) P. Beauv.)

Sweet grass (SG)**,** also known as manna grass, or Mary's grass, is an aromatic herb native to northern Eurasia and North America, which belongs to the family *Graminaeae*. It is considered sacred by many indigenous peoples in Canada and the United States. It has been used in a folk medicine as a smudge, and in the production of distilled beverages. SG is harvested by cutting grass at the desired length in early-to-late summer. It is denoted by a strong specific coumarin(1,2-benzopyrone)-like aroma (with ethanol extracts containing 3.57% (w/w) of coumarin and aerial parts 3.72% (w/w)) (Ueyama *et al*.[74]), and therefore it may be an undesired ingredient in food products. Even more, the levels of the tolerable daily intake (TDI) of coumarin are restricted to 0.1 mg/kg body weight (EFSA[75]) as it has toxic, cytotoxic, and carcinogenic potential; it also involves a protective effect (EFSA[76]). Coumarins are a group of natural phenolic compounds which are present in a variety of higher plants. A broad range of structures of coumarins have been isolated from different natural sources (fruits, vegetables, green tea), and some have been synthesized (Bilgin *et al*.[77]; Hoult *et al*.[78]).

Natural antioxidants are preferred to synthetic ones. A strong antioxidant activity of sweet grass extracts was reported for the first time in 2000 by Bandonienė *et al*.[79], giving an impact for examining it's properties against lipid oxidation in another foodstuffs. Pukalskas *et al*.[80] identified 5-hydroxy-8-*O*-beta-D-glucopyranosyl-benzopyranone and 5,8-dihydroxycoumarin in sweet grass, which are two main antioxidants. 5,8-dihydroxycoumarin (**Fig. 1.4.**) found in sweet grass was discovered to exhibit a greater antioxidant activity as compared to rosmarinic acid, a well-known natural antioxidant. Slapšytė *et al*.[81] isolated 5,8-dihydroxycoumarin from aerial parts of sweet grass and determined 5,8-DHC to be highly efficient in retarding rapeseed oil oxidation.

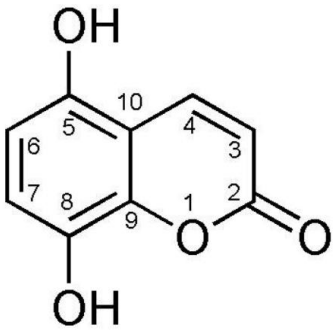

Fig. 1.4. Chemical structure of 5,8-dihydroxycoumarin

Grigonis *et al*.[82] investigated the extraction of strong antioxidants 5,8-dihydroxycoumarin and 5-hydroxy-8-O-beta-D-glucopyranosyl-benzopyranone

from sweet grass by using green extraction methods including *supercritical fluid carbon dioxide* (SFE), *microwave-assisted* (MAE) and *Soxhlet* (SE) extraction. The most concentrated extract, with 8.15% of 5,8-dihydroxycoumarin (extract yield of 0.42%), was produced via one-step MAE. To boost the concentration of active chemicals, a two-step extraction process was used. Their study demonstrates that antioxidants from sweet grass may be extracted by using both MAE and SFE.

Still, the scientific literature on the properties, chemical composition and usage of sweet grass in food is scarce. Mainly, sweet grass was tested in rapeseed oil, its emulsions, and pork lard (Bandonien *et al*.[79]; Zainuddin *et al*.[83]), where the extracts from SG even at very low concentrations (0.02%) were found to be effective natural antioxidants. It gave the same effect on accelerated oxidation conditions on the stability of rapeseed oil as butylated hydroxutoluene (BHT) at the same concentration.

1.3.3. Hemp (*Cannabis sativa* L.)

An important trend in the development of meat products is the use of cheaper plant-based protein substitutes in their composition (Sha and Xiong[12]). Various plant origin proteinaceous ingredients, including industrial hemp, have been gaining popularity, and are being tested in meat products after favourable regulatory changes for their wider cultivation (Crini *et al*.[84]). For example, Zahari *et al*.[85] developed meat substitutes with hemp protein by using extrusion cooking. Although, the use of hemp is still rather scarce.

Cannabis sativa is a herbaceous plant original to Eastern Asia, but now widespread. Hemp is one of the oldest and most widely grown crops in the world (Wang *et al*.[86]). It is a multipurpose crop, used as a source of industrial fibre, high-value seed oil (approximately 30%), food, religious purposes, and medicine. Traditionally, hemp seeds for fibre production were considered as a waste or mainly used as animal feed. Due to their nutritional features and health benefits, the production of hemp seeds has been increasing. Hemp seeds are sources of high quality easily digested proteins (25–30%), carbohydrates (20–30%), from which around 98% dietary fibre (mainly insoluble), vitamins (mainly, vitamin E, 90 mg/100 g), and minerals (Fe, Mn, Zn, and Cu) (Koziol[87]; Montero *et al*.[88]); it is also an excellent source of high-value oil (hempseed fat), known for its richness in polyunsaturated fatty acids (Pihlanto *et al*.[24]; Teh and Birch[89]; Xu *et al*.[90]). The oil content of hempseed ranges from 25–35% of the whole seed (Farinon *et al*.[91]). Due to its nutritional value, the hemp seed has become a novel food ingredient. The quality of hempseed protein is comparable to egg white (Koziol[87]). The crude protein concentration of fresh whole hempseed products ranges between 21.3–27.5%, between 30.3–38.7% in dehulled hemp seeds, and between 31–53.3% in hempseed meal (House *et al*.[92]). Hempseed protein mainly consists of albumin, legumin and arginin (Chen *et al*.[93]). On the other hand, the extensive use of hemp seeds in the food industry has been hampered by the high level of tetrahydrocannabinol (THC) in hemp seeds, which can neurologically change a person's sensory and psychological experiences and may eventually result in central

nervous system depression (Potin and Saurel[94]). Around 20 years ago, industrial hemp with a 0.3% lower THC level became available.

However, due to the high protein content, mechanically pressed hempseed press-cakes still contain approximately 10% highly unsaturated residual oil, which is sensitive to oxidation. Therefore, when using press-cake in meat products, the oil should be removed by extraction.

There has been only limited research on the phenolic content of hemp seeds, but the main phenolic compounds in cannabis seeds are lignanamides, hydroxycinnamic acids, hydroxybenzoic acids, and flavonoids (Leonard *et al*.[95]). Lignanamides display anti-inflammatory and antioxidant capacity. Many of the phenolic compounds have shown good *in vitro* antioxidant activity and are considered to possess various beneficial health effects, *e.g.* a reduction of the risk of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes and osteoporosis. In addition, hemp seeds contain bioactive peptides (Farinon *et al*.[91]; Martinez *et al*.[96]). TPC of hemp seeds cultivated in Italy was 2.33±0.07mg *Gallic Acid Equivalent* (GAE)/g *dry weight* (DW), of course, depending on the cultivar and the part of the hemp seed (Montero *et al*.[83]). Pihlanto *et al*.[24], also confirmed by Rea *et al*.[97], stated the total phenolic content of dry defatted hempseed flour to measure at 5.88–10.63 (mg GAE/g).

Hemp also contains cannabinoids and terpenoids. Non-psychoactive cannabinoid cannabidiol (CBD) has gained interest due to having a positive effect on human health (Pellati *et al*.[98]). Industrial hemp seeds also contain a small amount of tetrahydrocannabinol (THC).

Kotecka-Majchrzak *et al*.[7] investigated the effect of the addition of hemp cake on the physicochemical, textural, and sensory properties, and on the lipid oxidation of meatballs (vacuum packed and cooked) during storage at low temperatures. The supplementation of hemp cake (7.4%) improved the volume of dry matter and decreased the water content. With higher doses of hemp, the results for lightness (L^*) and redness (a^*) values were significantly decreased. Unlike changes in colour, pH-values did not change significantly throughout the 12 days of storage, regardless of the amount of hemp additive upon the addition of hemp. Hemp cake considerably reduced protein and lipid oxidation. According to sensory analysis, meatballs made with 0.9% and 2.6% hemp cake received superior overall ratings. The findings suggest that hemp cake, which is a by-product of cold pressing of hempseed oil, a substance typically regarded as waste or by-product, may be used in food as an alternative and nutritious ingredient in the production of sustainable meat products. Besides, Naumova *et al*.[99] point out that hemp flour protein in cutlets does not affect adversely its quality parameters.

1.3.4. Rowanberry (*Sorbus aucuparia* L.)

Sorbus aucuparia (The European rowan), also known as rowan or mountain-ash, belongs to the genus *Sorbus* of the rose family, *Rosaceae*. Fruits are 4–8 mm in diameter, orange or red in most species, but pink, yellow or white in some Asian species. Traditionally, its fruits are used as diuretic, laxative, anti-inflammatory

agents and for the treatment of various gastrointestinal disorders (Bobinaitė *et al.*[100]; Shikov *et al.*[101]).

Rowan is used for making jelly, jams (Berna *et al.*[102]; Mrkonjic *et al.*[103]), it has many uses in alcoholic beverages, although, due to its astringent taste, it has limitations in applications to food. Though, there are some cultivated hybrids, such as Likernaja, which are denoted by a less astringent taste. Therefore, it is important to know the acceptable dose to be added, for instance, in meatballs, in order to achieve the proper sensory properties as *Sorbus* still remains an underutilised genus. Jam from rowanberries has been considered as a proper addition for meat dishes (Hallmann *et al.*[104]). According to Bobinaitė *et al.*[100], rowanberry pomace contains 10.04% of protein, 19.57% of crude fibre, fat content 6.15%, and 6.29% of sugars. Based on the berry genotype and technological treatments during juice pressing, the chemical composition can vary. After SFE-CO_2 extraction (removal of the lipophilic fraction), the content of proteins, crude fibre and sugars in pomace residue was slightly higher than in the initial material.

A number of phytochemicals have been identified in various *Sorbus* spp. (Sołtys *et al.*[105]). The presence of significant amounts of polyphenolic antioxidants, mainly flavonoids and phenolic acids, has also been reported in *Sorbus* spp. The main phenolic acids are chlorogenic (3-*O*-caffeoylquinic acid, 3-CQA) and neochlorogenic acids (5-*O*-caffeoylquinic acid, 5-CQA) (Olszewska *et al.*[106]; Becerra-Herrera *et al.*[107]), which contribute to up to 80% of the total phenolics in wild and cultivated rowanberries (Kylli *et al.*[108]). Chlorogenic acids (**Fig. 1.5.**) occur according to the cultivar between 56–80% of the total amount of all polyphenols (Kylli *et al.*[108]).

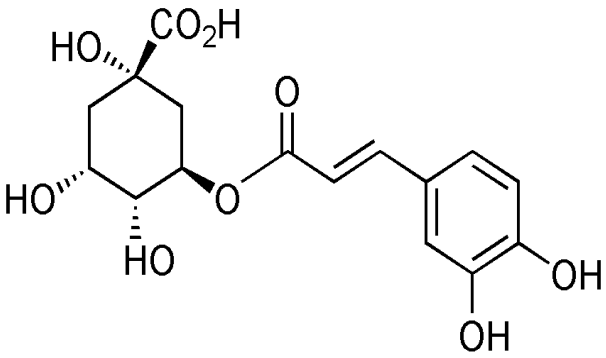

Fig. 1.5. Chlorogenic acid

Poyrazoglu[109] reported a 3-fold higher amount of ascorbic acid in rowanberry than in oranges. Polyphenolic compounds and ascorbic acid may be considered as the most valuable health beneficial constituents. The main polyphenolic compounds responsible for antioxidant properties of rowanberries are phenolic acids (mostly, caffeoylquinic acids), flavonols (quercetin, isoquercetin, hyperoside, rutin, catechin, epicatechin), and anthocyanins (mainuly, cyanidin or pelargonidin glycosides).

The latter are mainly found in the cultivated rowanberry species, whereas, in wild rowanberries, they are only found in low amounts (Kylli *et al*.[108]). Rowanberry fruits are also rich in carotenoids.

Sarv *et al*.[110,111] and Kylli *et al*.[108] reported the antioxidant and bacteriostatic effects of rowanberry extracts. Kähkönen *et al*.[112] found that even as little as 0.05% of rowanberry extract inhibited over 90% of the formation of methyl linoleate-conjugated diene hydroperoxides. Fruits of *S. aucuparia* showed high scavenging of $DPPH^{\bullet}$ and lipid peroxidation inhibition compared to other species (Hukkanen *et al*.[113]). According to Sarv *et al*.[111], rowanberry pomace, especially the hybrid cultivars, compared to the rowanberry juice or fruits, exhibited an even higher average total phenolic content and antioxidant capacity. Rowanberry pomace is a promising natural compound with antioxidant and biological activities.

1.4. Meat Quality Characteristics

According to the Regulation of European Parliament and Council No. 853/2004/EU[114], meat is defined as edible parts of the animals, including blood. The term 'meat quality' is broad, as it covers a variety of characteristics. The definitions differ between scientific disciplines involved. A typical definition for meat quality is "the total of all characteristics of a product," or the "set of properties that together identify what we appreciate, when buying or eating the meat." Meat quality is often defined as the fresh-meat-eating quality, which describes the meat quality for further processing. The fact is that the meat quality is an important factor ensuring consumer gratification. The term 'quality' can indeed be subjective, and it varies among individuals and products. The perception of the meat quality is multifaceted.

From a consumer's perspective, quality is often associated with the perception of a product being worth the price paid. Different people may have different criteria and expectations when evaluating the quality of meat. With the increasing demand for RTE foods and globalization, concerns about the food quality and safety are also increasing. When it comes to evaluating the meat quality, there are several characteristics that are commonly considered, along with several factors affecting its quality. Parameters of the meat quality can be divided into 4 groups (**Fig. 1.6.**):

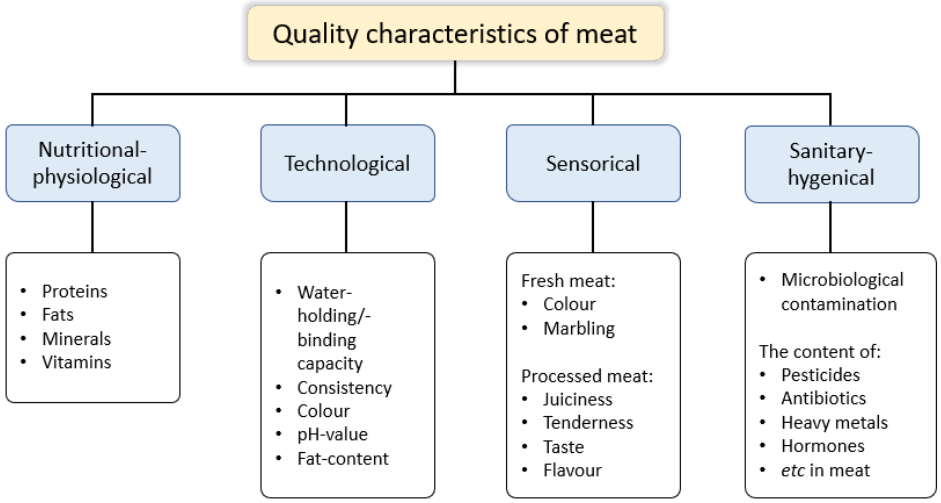

Fig. 1.6. Categorization of meat quality characteristics (the figure is composed based on Eesti Toiduainete tehnoloogia Selts[115])

The chemical composition of meat, besides the main physical properties, determines its quality. The chemical composition of meat is quite variable depending on the type of meat, the breed, the age of the animal, the physical load, and the feeding of the animal (Rei[116]). Meat consists of approximately 75% water, 19% protein, and 2.5% fat (Olaoye[117]). This chemical composition changes meat into a complex matrix that is susceptible to oxidative processes.

Traditionally, the characteristics relating to our sensory experience have been used to determine the quality of meat intended for consumption: its appearance, colour, flavour, texture (particularly tenderness), juiciness/water-holding capacity, and odour. Meat quality preferences among consumers (and hence its market value) are typically based on its colour, marbling, texture, and juiciness.

pH is a numerical indicator of how acidic or basic aqueous or other liquid solutions are. The pH level, which is closely related to the colour of the meat, tenderness, drip loss, and eating quality attributes (toughness after cooking), is the most crucial meat quality indicator. The ideal range of pH for pork is 5.8 to 6.2. It is well known that measuring pH is crucial for predicting the growth of microorganisms. Most bacteria grow at pH-level 7, and the higher is the final pH of the product, the shorter is the shelf-life (Sallam *et al*.[118]).

It has been noted that when meat is packaged under modified atmosphere (MAP), including CO_2, the pH of the meat falls due to some of the CO_2 partial absorbance into the meat (Jakobsen and Bertelsen[119]).

For the growth of microorganisms, the presence of free water is necessary. Water activity (a_w) is the ratio of the vapour pressure in a food to the vapour pressure of pure water. Water activity is used in the formulation of shelf-stable foods

by virtue of being one of the quality characteristics. Mould growth is prevented if a product is kept below a specific water activity level. As a result, the shelf life is extended. At a_w less than 0.75, microorganisms cannot grow, whereas, for most, the development is retarded at 0.91. The majority of bacteria can develop in fresh meat at a_w 0.99. The growth of microorganisms is optimal at pH 7.0 (neutral). In general, at a pH-level between 6.0 and 8.0, bacteria grow the fastest, while this value stands for yeasts at 4.5–6.0, and for filamentous fungi at 3.5–4.0 (Warris[120]).

The ability of meat to hold or bind the water it contains during processing (cutting, heat treatment, mincing, or other mechanical processing) is referred to as its 'water-binding capacity'. Numerous chemical, physical, organoleptic, and technological characteristics of meat and meat products are influenced by the ability of flesh to bind water. It includes colour, dullness, juiciness, tenderness, texture, product yield, and the structure of the raw meat. The hardness and ability of meat to hold water are related to the drip loss. A high drip loss can cause excessive cooking losses and drying of the meat. Water is located in several different places in the muscle, and water loss from different locations in the muscle may depend on the storage time and the location of water.

The colour of meat as a sensory characteristic is the first indicator which consumers tend to detect; on the other hand, every person perceives it differently. The colour of meat depends on several factors: the species, age, type of a muscle, etc. Moreover, it can be influenced by the important quality parameter, notably, pH. There are several crucial activities that occur in meat during processing and storage that are related with the colour qualities. The bright cherry-red colour is usually an indicator of freshness of the meat. Two proteins, haemoglobin and myoglobin, give meat its colour. As the flesh is usually reddish, it reflects the long-wavelength red light to the human eye while absorbing other colours. To describe the colours visible to the human eye, the CIELAB system (the L^*, a^*, b^* colour space scheme) is used, which characterizes colours according to their hue (a^* – redness-greenness, b^* – yellowness-blueness, and lightness – L^*) (MacDougall[121]). In addition, packaging has an effect on the colour as well. Nitrogen in *modified atmosphere packaging* (MAP) has been observed to improve the colour, *e.g.*, in meat loaves, by inhibiting the development of the green colour. Therefore, it helps to stabilize the colour (Narasimha Rao and Sachindra[122]). The colour and sensory parameters of meat products have been found to be either positively or negatively impacted by the addition of various natural antioxidants; however, the effect of plant-extracts used depends on the extraction method, the treatment of the samples, and the treatment of post-packaging.

Flavour is another main attribute after colour, which determines the consumers' purchase decisions. Flavour is a combination of taste and odour.

The meat structure and its chemical composition are affected by heat treatment contributing to the development of specific sensory properties (texture, taste and colour) and making the products tastier and more appetizing for consumers. Heat treatment launches different reactions, like *Maillard* reaction, lipid oxidation, which result in the development of the characteristic flavour of meat. During thermal processing, thousands of volatile compounds are generated, and the species-specific

flavour of meat is determined by mixtures of volatile compounds. The final composition of volatile compounds depends also on the other ingredients added (Kosowska *et al*.[123]). While cooking, the colour of the meat will turn brownish or greyish-brown due to *Maillard* reaction, when free amino group containing compounds (*e.g.*, amino acids, peptides, proteins) and certain reducing sugars (*e.g.*, glucose, ribose) are reacting under the influence of temperature, and the so-called *Maillard* reaction products are formed. During the reaction, the surface of the food turns brown, and a crust is formed, the taste of the product changes, and the characteristic flavour is created. The *Maillard* reaction proceeds very slowly at low temperatures, while its rate remarkably increases at >140 °C (Tamanna *et al*.[124]). Cooking loss is another quality parameter, depending on the quality of the flesh and the method of heat treatment of the meat. Cooking loss by itself affects another sensory quality attribute, that is, the juiciness and the appearance of the product. For the meat industries, it has an economic effect (Aaslyng *et al*.[125]).

The texture of the meat depends on the age, gender, breed of the animal, and the type of the muscle. By texture indicators, we understand hardness (toughness), springiness, chewiness, and sometimes also juiciness. Consumer surveys have shown that the most crucial aspect of the meat eating quality is tenderness followed by juiciness, which are affected by pH, the fat content, and the cooking method used.

Meat is a very complicated matrix, and, before heat treatment, it is 'alive'. Therefore, different reactions and processes occur affecting its quality negatively. One of those processes is oxidation. Lack of antioxidants, a high content of PUFAs, heat and light during processing etc., all of these promote oxidation in meat. Lipids are vital from the human nutrition point of view. Lipid oxidation is catalyzed as the presence of ROS, RNS and metallic ions. Lipid oxidation strongly affects lipids, proteins, pigments, vitamins, while also causing sensory degradation. Losses in the nutritional value lead to the formation of toxic substances; therefore, controlling oxidative processes is crucial for the meat industries. During lipid oxidation, unsaturated fatty acids react via the free radical mechanism with oxygen and produce hydroperoxides as the first oxidation products. However, due to their instability, these substances quickly break down into a variety of secondary substances, such as acids, alcohols, esters, hydrocarbons, aldehydes, and ketones (Ross and Smith[126]), thereby causing off-flavours and off-odours in meat. Therefore, prevention of lipid oxidation in meat is significant from the meat quality and human health perspective (Huang and Hahn[127]). Lipid oxidation is a complex process which depends on the chemical composition of the meat, light, oxygen, and the storage temperature. Lipid oxidation can be measured by the amount of the peroxide value (primary lipid oxidation products), by headspace hexanal (the most abundant secondary lipid oxidation product), thiobarbituric acid reactive substances (TBARS) (secondary lipid oxidation products), sulphyric and carbonyl groups produced during the storage (Lorenzo *et al*.[34]). Malondialdehyde (MDA; $CH_2(CHO)_2$), as a highly toxic substance, is one of the final products of oxidation, known as a marker of oxidation. Therefore, it is essential to control its level. In addition, sensory methods

can be used by detecting the levels of oxidation perceivable by humans (Estévez *et al*.[128]).

In order to inhibit the oxidation of lipids, antioxidants and bioactive compounds are added directly into the product, on the surface of the product, or inside the packaging material (Nikmaram *et al*.[36]). Antioxidants are compounds capable of dispensing hydrogen radicals to bind to other free radicals, and thereby they prevent the reproduction reaction during the oxidation process (Masuda, Inaba and Takeda[129]). Phenolic compounds can function as primary antioxidants as chain breakers, by reacting with free radicals generated during lipid oxidation.

Antioxidants used in food must meet a number of requirements:

- must not have any negative effect on the odour, taste or colour of the product;
- must be effective at low concentrations (0.001–0.01%);
- must be compatible with food;
- must maintain stability;
- must be economically feasible (*i.e.*, profitable);
- must not be toxic in quantities greater than those obtained with normal food (Lorenzo *et al*.[34]).

The effectiveness of antioxidants depends on the concentration, temperature, pH, processing conditions, molecular structure, and polarity (Nikmaram *et al*.[36]).

1.5. Extraction Methods

Humankind has extracted useful compounds from natural resources already centuries ago. Records show that, between 500 and 400 BC, in Ancient Greece, essential oils were extracted from aromatic food plants, such as peppermint, saffron, thyme, cumin, and marjoram (Elshafie and Camele[130]). Enfleurage extraction, a technique used during ancient times, was used to extract volatile compounds by using pure and odourless cold fat. Later, the extraction technique known as hydro-distillation was developed in order to recover the essential oils more efficiently. The traditional method for recovering bioactive chemicals from plant materials is maceration, which is based on the leaching of compounds from a solid material (Naviglio *et al*.[131]). Various extraction methods used are presented in **Fig. 1.7.**

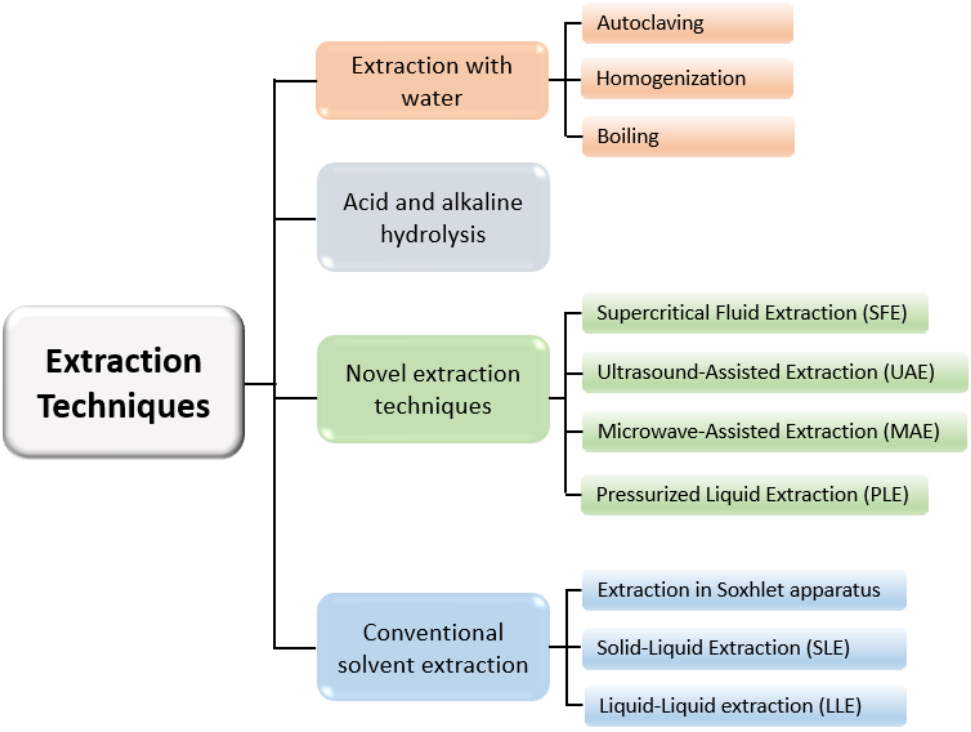

Fig. 1.7. Various extraction techniques

In order to extract useful molecules from raw materials, such as plants, without significantly harming the environment or using an excessive amount of energy and resources, so-called 'green extraction methods' are being developed, and some of them are already in use (Koina *et al.*[132]). With these techniques, less dangerous solvents are being used, they are more sustainable and economic, an increase in the use of renewable energy sources is achieved along with a decrease in the use of toxic and dangerous chemicals and an improvement in human health and life quality. These are some of the positive effects of green chemistry methods (Anastas and Eghbali[133]). Aqueous-based techniques are promising since water is non-toxic, non-flammable, and cheap. On the other hand, it has low solubility of apolar compounds. The recovery of natural compounds, rich in phytochemicals, meant for the food industry has been significantly impacted by the innovative development of green chemistry methods. Green chemistry and the increased public interest in chemical-free products have jointly driven the innovation in the green extraction methods. Based on the effectiveness of extraction and the use of a solvent and energy, the extraction techniques can be categorized into broader groups, such as the conventional extraction methods including maceration, hydrodistillation, solvent extraction, and green extraction techniques including microwave and ultrasound-assisted extraction, supercritical fluid extraction, and pressurized liquid extraction.

These technologies greatly increase the extraction efficiency and yield while using less solvent and consuming less energy (Awad *et al.*[134]; Pateiro *et al.*[135]; Picot-Allain *et al.*[136]). A comparison of the conventional and green extraction methods used to extract bioactive compounds from plant matter according to Awad *et al.*[134] is shown in **Tbl. 1.2**.

Table 1.2. A comparison of green and conventional extraction technologies (Awad *et al.*[134])

Green extraction methods	**Conventional extraction methods**
Lower energy consumption during extraction process	Higher energy consumption during extraction process
Low solvent utilization	Higher use of solvent
Higher recycling and reusability of solvent	Lower chances to reuse of recycled solvent
Very low or zero leftover residue of solvent	Solvent leftover in higher amounts
Fast process	Slow process
High efficient process	Low efficient process
Simple and low cost	Complex and high cost process

For the past few years, various berry pomaces have been proposed and tested by using an effective green consecutive extraction platform with increasing polarity solvents (Bobinaitė *et al.*[100]; Kitrytė *et al.*[137]; Tamkutė *et al.*[138]). Polar compounds can be extracted by using polar solvents (*e.g.*, water and ethanol), whereas hydrophobic compounds are best extracted by using organic solvents (hexane, ether, methanol). To reduce the impact of organic waste on the environment and to increase the efficiency of extraction, the solvents used for extraction should be nontoxic, reusable, easy to remove from extracts, inexpensive, and conveniently accessible (Birla *et al.*[139]; Rathour *et al.*[140]). In order to extract bioactive compounds from plant materials, green solvents, such as water, supercritical fluids, synthetic ionic fluids, and ethanol are being used ever more frequently (Beya *et al.*[141]; Pateiro *et al.*[135]). It is hypothesized by Basegmez *et al.*[142] that the use of novel extraction and fractionation methods for biorefining blackcurrant pomace into various products could significantly improve the efficiency of processing, especially when developing ingredients with high added value for food.

Advantages and disadvantages of novel extraction methods are presented in **Tbl. 1.3.**

Table 1.3. Advantages and disadvantages of various extraction methods

Extraction method	**Pros**	**Cons**
SFE	1. Efficient and economic (Ibañez *et al.*[143]) 2. Minimum use of organic solvents (Kadam *et al.*[144]) 3. Environmentally friendly (Kadam *et al.*[144]; Ibañez *et al.*[143]; Halim *et al.*[145])	1. Costly procedure (Kadam *et al.*[144]) 2. Because of its non-polar nature, sc-CO_2 will not extract polar

Extraction method	Pros	Cons
	4. Industrial scale application (Ibañez *et al.*[143]) 5. Extraction of bioactive compounds without the loss of volatility (Kadam *et al.*[144]) 6. Heavy metals and inorganic salts free extract (Hosikian *et al.*[146])	substances (Hosikian *et al.*[146]) 3. More intensive labour is required for sample processing (Klejdus *et al.*[149])
MAE	1. Low extraction time (Hahn *et al.*[147]) 2. Improved and fast extraction (Ibañez *et al.*[143]; Kadam *et al.*[144]) 3. Low amount of solvents are required (Ibañez *et al.*[143]; Kadam *et al.*[144]) 4. Higher yields of extracted compounds (Kadam *et al.*[144]) 5. Capable to couple reaction and extraction at the same time (Ibañez *et al.*[143]) 6. Cost-efficient (compared to conventional extractions) (Kadam *et al.*[144])	1. Energy input-assisted extraction (Hahn *et al.*[147]) 2. Additional separation process needed to remove solid residues (Hahn *et al.*[147]) 3. Not suitable for heat-sensitive bioactive compounds (Hahn *et al.*[147])
UAE	1. Simple and economic technique (Kadam *et al.*[144]; Ibañez *et al.*[143]) 2. Low amount of solvents is needed (Ibañez *et al.*[143]) 3. Improved extraction yields (Kadam *et al.*[144]) 4. Extraction of heat-sensitive compounds with minimal damage (Kadam *et al.*[144]) 5. Easy to combine with other extraction techniques (Kadam *et al.*[144])	1. High amount of energy applied may degrade bioactives (Hahn *et al.*[147])
PLE	1. Considerably small amount of solvents used (Kadam *et al.*[144]; Shang *et al.*[148]) 2. Faster than other solvent extractions (Shang *et al.*[148]) 3. Short extraction time (Shang *et al.*[148]) 4. High pressure and temperature (Shang *et al.*[148]) 5. A wide range of solvents can be used (Kadam *et al.*[144]) 6. High selectivity	1. Not suitable for heat sensitive substances (Ibañez *et al.*[143])

SFE – supercritical fluid extraction; MAE – microwave assisted extraction; UAE – ultrasound assisted extraction; PLE – pressurized liquid extraction

1.5.1. Supercritical fluid extraction with carbon dioxide (SFE-CO_2)

Supercritical fluid extraction (SFE) is a green technology using supercritical fluid solvent, mostly carbon dioxide (CO_2) or water, for the extraction. The SFE-CO_2 process combines extraction, separation and concentration to obtain the extract. When the substance's temperature and pressure are above the critical point, a supercritical fluid is produced, which is the basis for supercritical fluid extraction.

The critical temperature and the critical pressure for SFE-CO_2 are higher than 31 °C and 74 bar, respectively (Awad *et al.*[134]; Belwal *et al.*[150]). **Fig. 1.8.** represents the SFE extraction unit, which should include a tank for CO_2 as a mobile phase, a pump for CO_2, an extraction vessel with a heating jacket, a sample-collecting vessel, and a pressure controller. Liquid CO_2 is pumped into the heating zone and heated to supercritical conditions. Then, in the extraction vessel, it diffuses into a solid matrix and dissolves the material which is to be extracted. Dissolved compounds are transported from the extraction cell into the separator. Finally, the extract is collected, and CO_2 can be cooled, recompressed, recycled, or released to the atmosphere. This method has been used in the production of food and feed additives, nutraceuticals. In the food industry, its main applications are coffee decaffeination, as well as extraction of hemp and other plant extracts.

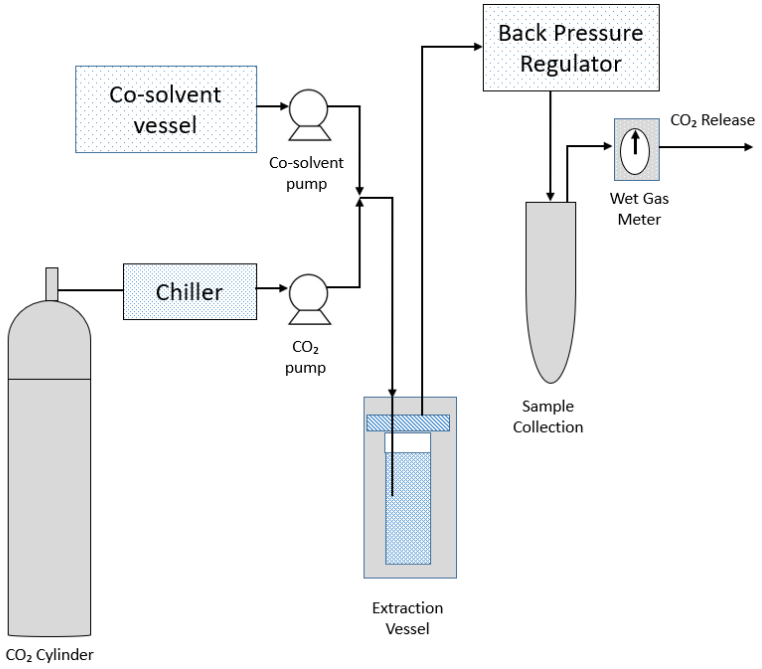

Fig. 1.8. Supercritical fluid (SFE) extraction unit (figure is modified from Hartati *et al.*[151])

CO_2 as a solvent is *Generally Recognized As Safe* (GRAS) (Wrona *et al.*[152]); this is an effective solvent for botanicals; a small amount of sample can be used, and it can be easily removed from the extracts without any losses of volatiles, compared to the conventional methods (Hosikian *et al.*[146]). scCO_2 is a good solvent for the extraction of non-polar compounds: essential oils, fats, tocopherols, carotenoids, etc., but phenolics, alkaloids, and glycosidic compounds are almost insoluble in it due to the high molecular weight. Polar co-solvents (modifiers) are effectively used to increase the solubility of those polar compounds. For any plant-based material which has a high water content, and which is not soluble, it is better to use dried material. This extraction method provides a broad range of useful properties, *e.g.*, elimination of the use of organic solvents (posing storage and environmental

problems). Carbon dioxide has a number of benefits because it is harmless, inexpensive, and readily available in its pure form. Processing at low temperatures ensures that heat-sensitive compounds are preserved, and that the recovered compounds are of a higher quality and better functionality (Khaw *et al.*[153]). Disadvantages of SFE are the operation at high pressures; it also requires investment into the equipment, and polar compounds are insoluble in CO_2.

Gallo *et al.*[154] reported SFE to increase the protective effect of plant extracts against lipid oxidation in cooked chicken meat. Besides, extracts of *Echinacea angustifolia* prevented lipid oxidation compared to the conventional extraction method.

1.5.2. Microwave-assisted extraction

Microwave is a radiation with wavelengths ranging from about 1 meter to 1 millimetre corresponding to frequencies between 300 MHz and 300 GHz, respectively. *Microwave-assisted extraction* (MAE) is a conventional technique for extracting active compounds from plants, where microwave energy is used to heat the solvents in contact with a sample and disrupt the cell membrane. Analytes are separated from the sample matrix and transferred into the solution; therefore, the yield of extraction increases, and the consumption of the solvent is decreased. The advantages of MAE is the quick heating of the sample solvent (as less energy is needed), its applicability for the rapid extraction, and the possibility to be used for thermally unstable substances. As the need for solvents is small, or as even there is no need to use any (dry MAE), it is considered as a 'green' technology. The key difference between MAE and the conventional extraction is that, in MAE, the gradient of the heat transfer is from the solid to the liquid phase, whereas, in conventionally heated extraction, the gradient is from the liquid to the solid phase (Périno-Issartier *et al.*[155]). Due to its properties, this extraction technique has recently been employed for the recovery of numerous bioactive compounds, including polyphenols.

MAE has been mainly used for the extraction of polysaccharides and fats, pigments, phenols, phytosterols (Michalak and Chojnacka[156]). The use for extracting bioactives from plants and herbs has been common due to its advantages (Cintas *et al.*[157]). According to Kadam *et al.*[144], compared to SFE, microwave assisted extraction is more cost-effective. On the other hand, heat-sensitive bioactives are not suitable for MAE.

MAE has been previously used to obtain polyphenolic compounds from tomato peels. Bakic *et al.*[158] investigated the impact of various solvents in MAE and found that 70% (v/v) methanol with 1% (v/v) HCl at 90 °C produced the best yields for total phenols, total flavonoids, and specific phenolic compounds. Solaberrieta *et al.*[159] investigated the effect of MAE and ultrasound-assisted extraction on the recovery of antioxidants from tomato seed waste. In their work, MAE and ultrasonic-assisted extraction of antioxidants were optimized by using a response surface methodology. In order to extract bioactive compounds from tomato seeds with a strong antioxidant activity, MAE demonstrated itself as an efficient green technique with a significant potential for scaling up for the valorization of tomato

seed industrial wastes. Kurtulbaş *et al.*[160] extracted high added-value compounds from peach peels by using MAE.

1.6. Metabolomics

The first metabolomic study was reported in the 1970s by Horning and Horning[161]; the concepts were defined in 1999 by Jeremy Nicholson, who is titled as a pioneer in NMR-based metabonomics (Nicholson *et al.*[162]), and by Oliver Fiehn[163]. This technique was originally defined as a comprehensive analysis, comprising the quantification and identification of as many metabolites as possible in a biological system.

Metabolomics is a study of small molecules (metabolites; molecular weight <1000 Da), found inside cells, tissues or organisms, and involved in biological processes (Ramanathan *et al.*[164]). Those small molecules are, for instance, lipids, fatty acids, or phenolic compounds. Metabolomics is used in pharmaceuticals or agriculture, and the importance of this technique has also gradually increased in the food industry. In the past five years, many articles have been published describing the use of metabolomics in meat. It has been integrated for determining the qualitative characteristics or biomarkers of meat and meat products (Capozzi *et al.*[165]; Rocchetti *et al.*[6]) because it helps to expose a wide number of compounds related to the oxidation of meat products; it is recognized as a useful tool evaluating the overall meat quality (Agin *et al.*[166]). New analytical tools and metabolite databases make it possible to analyze thousands of metabolites and find novel metabolites that are present in food (Beale *et al.*[167]). According to Rochetti *et al.*[6], non-targeted metabolomics enables the identification of unknown molecules pertinent to food systems and the identification of the indicators of deterioration. In particular, the main targets for lipids oxidation are polyunsaturated fatty acids (PUFA) and phospholipids. Generally, oxidation processes are categorized into nonenzymatic and enzymatic oxidation. Nonenzymatic oxidation can be further divided into autoxidation (mediated by free radicals) and photooxidation (promoted by ultraviolet or singlet oxygen).

Metabolomic markers can even be used for predicting the pH of the meat (Beauclercq *et al.*[168]), to estimate flavour precursors, or characterize off-flavours in meat (Wang *et al.*[169]), or even to use in meat authentication by assessing and characterizing its chemical composition and metabolite contents (Zhang *et al.*[170]). The number of metabolomic research for meat tenderness has been used, but it is limited.

In order to systematically characterize the complex meat matrices, meat metabolomics is anticipated to become a powerful tool in quality analysis and authentication. Still, the use of metabolomics in routine analyses of the meat quality remains limited, and there is a need to develop harmonized and normalized sampling methods.

2. MATERIALS AND METHODS

This chapter quotes passages from the articles with the permission of *Foods*[1] and *Elsevier*[2,3] journals.

The research consists of two main parts: the preparation of plant-based ingredients, and the evaluation of their effect on the quality characteristics of meat products. The principal scheme of the three experiments is presented in **Fig. 2.1.**

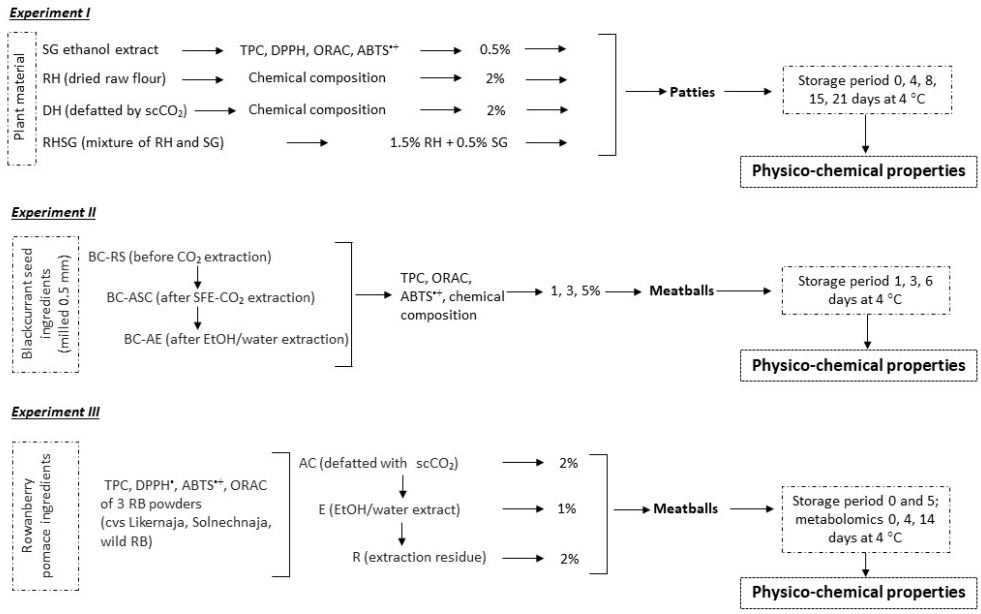

Fig. 2.1. The principal scheme of the research

2.1. Research Objects and their Pre-Treatment

Dried and mechanically pressed hempseed cake was obtained from the company *Agropro* (Kaunas, Lithuania).

Dried sweet grass (*Hierochloe odorata* (L.) P. Beauv.) was purchased from the company *Mėta* (Vaidotai, Vilnius, Lithuania).

Blackcurrant pomace seeds, dried and mechanically separated from the skins, were from one batch in 2022, and donated by the Cooperative *Ribes LT* (Biržai, Lithuania). The skins and seeds were from the same blackcurrant pomace.

[1] Application of Raw and Defatted by Supercritical CO_2 Hemp Seed Press-Cake and Sweet Grass Antioxidant Extract in Pork Burger Patties. https://doi.org/10.3390/foods10081904

[2] Evaluation of different blackcurrant seed ingredients in meatballs by using conventional quality assessment and untargeted metabolomics. https://doi.org/10.1016/j.meatsci.2023.109160

[3] Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs. https://doi.org/10.1016/j.fochx.2023.100761

Rowanberry fruits were harvested in autumn 2021 from the experimental orchard of the *Polli Horticultural Research Centre* of E*stonian University of Life Sciences* (South Estonia, 58°07'44.5"N, 25°32'16.8"E), from which, the pomace was separated after juice extraction. Fruits were immediately frozen after the harvest and stored at -20 °C. Before extracting the juice with a juicer *Smeg SJF01CREU* (*Smeg S.p.A*, Guastalla, Italy), the fruits were defrosted.

Fresh pork minced meat for the experiments was purchased from a local commercial abattoir (Tartu, Estonia) and stored until use in the fridge below 4 °C.

2.2. Chemicals and Reagents

2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS$^{\bullet+}$); 2,2-diphenyl-1-picrylhydrazyl hydrate free radical (DPPH$^{\bullet}$, 95%); gallic acid (GA, 3,4,5-trihydroxybenzoic acid, 99%); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%); and Na_2CO_3 were purchased from *Sigma-Aldrich* (Steinheim, Germany). Folin–Ciocalteu phenolic reagent (2 M); 2-(3-hydroxy-6-oxo-xanthen-9-yl)benzoic acid (fluorescein); and 2,2′-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) were from *Fluka Analytical* (Bornem, Belgium). KCl, NaCl, $K_2S_2O_8$, and KH_2PO_4 were from *Lach-Ner* (Brno, Czech Republic). Na_2HPO_4 and isoamyl alcohol (a mixture of isomers) were from *Merck KGaA* (Darmstadt, Germany). Agricultural origin ethanol (96.3%) was from *Stumbras* (Kaunas, Lithuania). Liquid nitrogen was from *AGA SIA* (Riga, Latvia). CO_2 and N_2 gases (99.9%) were from *Gaschema* (Jonava District Municipality, Lithuania). Perchloric acid; 2-thiobarbituric acid; 1,1,3,3 tetraethoxypropane; butylated hydroxytoluene as well as sulfuric acid (H_2SO_4), boric acid (H_3BO_3) and hydrogen chloride (HCl; 0.561M) were purchased from *Sigma-Aldrich Chemie* (Steinheim, Germany). Sodium hydroxide (NaOH, 50%) was acquired from *Ingle AS* (Ingliste, Estonia), and Kjeltabs FOSS Analytical A/S was from *Oridor Eesti OÜ* (Tartu, Estonia). Sodium chloride (NaCl) (99.9%) for volatile aroma compounds was obtained from *Reachem s.r.b.* (Bratislava, Slovakia).

Ethanol (95% and 78%) was from Estonian *Spirit OÜ* (Tallinn, Estonia). Heat-stable α-amylase, amyloglucosidase, protease, analytical grade Celite and MES/TRIS buffer were from *Megazyme Ltd.* (Wicklow, Republic of Ireland). Acetone was from *Honeywell International Inc.* (Charlotte, NC, USA). The solvents used for UHPLC-HRMS analysis – water, acetonitrile, and formic acid (LC-MS grade) were purchased from *VWR* (Milan, Italy).

2.3. Characterization of Plant-Based Ingredients

2.3.1. Proximate analysis of blackcurrant ingredients

The proximate chemical composition of blackcurrant seed residues after all extractions was analyzed according to the standard methods: moisture (LST EN ISO 665:2020), protein (LST EN ISO 8968-1:2014, LST EN 12135:2001 and LST ISO 937:2000), fat (LST EN ISO 659:2000 (ISO 659:2009)), ash (ISO 749:1977), and mineral (LST ISO 936:2000) content. All analyses were carried out in four parallel replicates.

2.3.2. Extraction methods used for the preparation of plant-based ingredients

Part of the hemp press-cake was defatted by supercritical CO_2 extraction in a pilot 10 L extractor (*Applied Separations*, Allentown, PA, USA) at 350 MPa pressure and 60 °C temperature for 4 h, when the extraction kinetics curve reached the plateau.

High-pressure extraction procedures were applied to dried sweet grass with slight modifications. The herb was ground and extracted in a pilot-scale extractor with supercritical CO_2 at 40 MPa and 60 °C for removing lipophilic substances and volatile aroma constituents as SG possesses a strong specific aroma. Afterwards, the defatted and deodourized material was extracted with ethanol in an accelerated solvent extractor *ASE 350* (*Dionex*, Sunnyvale, CA, USA) at 10.3 MPa and 100 °C, using 3 cycles, 15 min each. The solvent was removed in a *Buchi* rotary vacuum evaporator (Flawil, Switzerland) at 40 °C.

The flowchart of BC seed ingredient preparation is shown in **Fig. 2.2.** The seeds were milled by using 0.5 mm sieve, followed by extraction with $scCO_2$ at previously optimized parameters (Basegmez *et al.*[142]). After $scCO_2$ extraction, lipophilic extract and defatted residue (BC-ASC) was obtained. Six portions of BC-ASC (100 g each) were mixed with 300 mL 96% ethanol in a 500 mL flask and extracted in a platform universal shaker *PSU20* (*Biosan*, Riga, Latvia) for 2 h at 155 rpm. The solid residues were re-extracted with 200 mL 96% ethanol. Then, the extracts were filtered in a Buchner funnel *Whatman* filter paper No. 1 (*Whatman International Ltd*., Maidstone, U.K.), combined and evaporated in a rotary vacuum evaporator *Rotavapor R-210* (*Büchi Labortechnik*, Flawil, Switzerland) at 40 °C. The yields from seeds and skins were 2.70 ± 0.13% and 3.46 ± 0.21%, respectively. The residue after ethanol extraction was dried at 40 °C, and 6 of its portions, 80 g each, were extracted with 400 mL of hot water in a shaker for 2 h. The residue was re-extracted 2 times with 200 and 150 mL of hot water. The extracts were decanted, combined, centrifuged in a refrigerated centrifuge *Velocity 18R* (*Dynamica Scientific Ltd.*, Livingston, UK) at −18 °C during 20 min and freeze-dried in a *Maxi Dry Lyo* (*Jonan Nordic A/S*, Aelleröd, Denmark). The yields from the seeds and skins were 7.22 ± 0.23% and 3.85 ± 0.19%, respectively. The residue after all extractions (BC-AE), as the third ingredient for the experiments, was obtained. Ethanol and water extracts are a good source of polyphenolic antioxidants, mainly anthocyanins; therefore, they may be used as promising ingredients in the formulation of health-beneficial products. As the main aim of the experiment was to test raw seeds and their by-products after different extractions, they were excluded from the experiments.

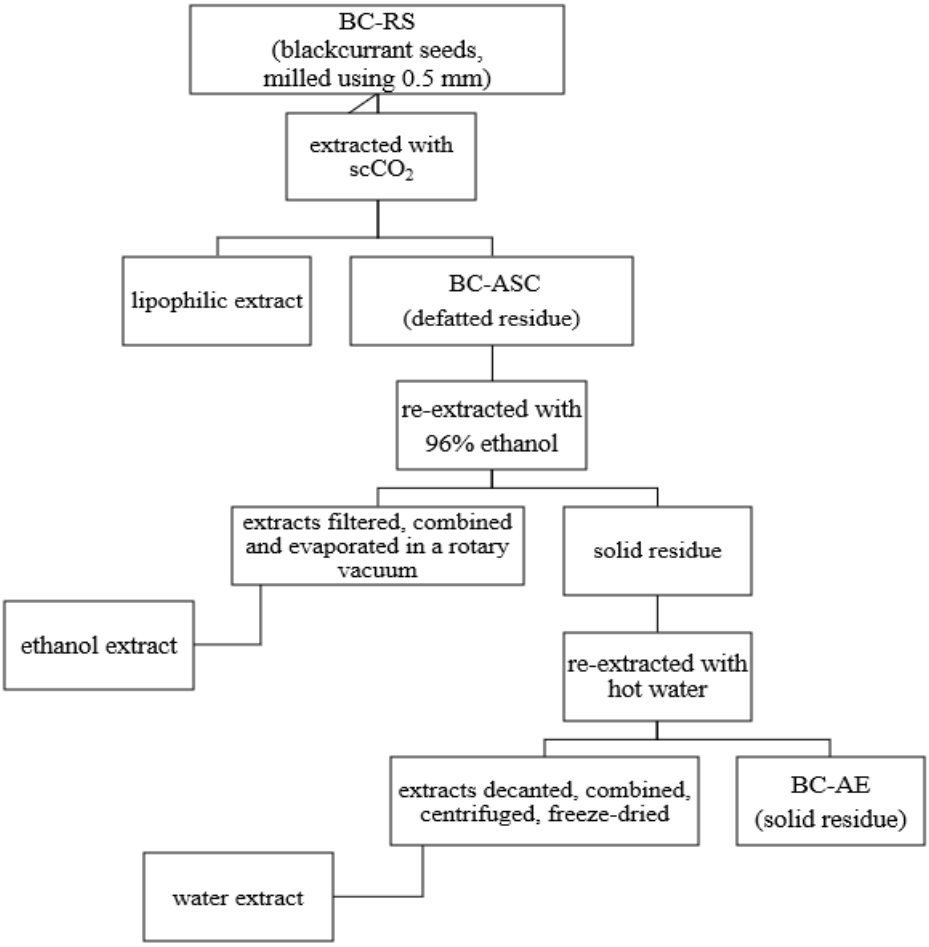

Fig. 2.2. Extraction scheme for BC seeds

After the above described extraction steps, the following BC ingredients were produced: BC seeds before supercritical CO_2 extraction (BC-RS), after $scCO_2$ extraction (BC-ASC), and after EtOH/water extraction (BC-AE).

For rowanberry, three pomace samples were ground in a *Retsch SM 300* cutting mill (*Retsch GmbH*, Haan, Germany), with sieve holes of a diameter of 5 μm. The obtained material was defatted with supercritical CO_2 extraction in SCF extraction equipment *Separex 5* (Champigneulles, France) for removing lipophilic substances at 40 MPa pressure at a temperature of 40 °C and in 150 min extraction time (Tamkutė *et al*.[171]). The first ingredient (AC) to be tested in pork meatballs was thus received. Due to the significant amount of polyunsaturated fatty acids found in berry seeds, which may hasten the development of oxidation products in meatballs

during storage, the lipophilic CO_2 extract was not used in the current research (Venskutonis[172]).

The first ingredient of rowanberry pomace, AC, was extracted with 1:1 (v/v) ethanol/water at a solid/liquid ratio of 1:10 (w/v) by using microwave assisted extraction (MAE) for 15 minutes at a power of 300 W followed by filtration. The EtOH part of the supernatant was dried in a rotary evaporator, while the remaining water was freeze-dried in a *VirTis Advantage Plus Benchtop Freeze Dryer Model XL-70* (*SP Industries*, Warminster, PA, USA). The freeze-dried extract (E), as the second ingredient, was stored in a sealed package in a freezer (-20 °C). The extraction residue (R), as the third ingredient of meatballs, was freeze-dried and stored in the grip seal polythene bag at room temperature.

2.4. Determination of Antioxidant Properties of Plant-Based Material

2.4.1. Total phenolic content (TPC)

The total phenolic content (TPC) was measured with Folin–Ciocalteu reagent (Singleton, Orthofer and Lamuela-Raventós[173]) with some modifications. Shortly, 30 μL of a sample (0.1%) was mixed with 150 μL of 10-fold diluted in distilled water Folin–Ciocalteu reagent and 120 μL of 7.5% Na_2CO_3 in microplate wells. After shaking for 30 s, the sample was incubated for 30 min at room temperature, and the absorbance was measured at 765 nm. For the BC solids, the QUENCHER approach (Kitrytė, Šaduikis and Venskutonis[174]) was adapted. The QUENCHER method is an extraction free method evaluating the total antioxidant capacity straight from the solid material, where some of the antioxidants may be bound and not extracted. Briefly, 150 μL of distilled water was mixed with 10 mg of the sample or cellulose (blank), 750 μL of Folin–Ciocalteu reagent, and 600 μL of Na_2CO_3 solution, then vortexed for 15 s and shaken at 250 rpm for 2 hours in the dark, followed by centrifugation (4500 rpm, 5 min). The absorbance was measured at 760 nm. The results were expressed in mg of GA equivalents per g of dry extract (E) weight (mg GAE/g dw).

2.4.2. $ABTS^{\bullet+}$ scavenging assay

$ABTS^{\bullet+}$ (2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid)) (**Fig. 2.3**) decolouration assay was measured according to a slightly modified method of Re *et al.*[175]. Pomace or cellulose (blank) were mixed with 25 μL of MeOH and 1500 μL of working $ABTS^{\bullet+}$ solution, vortexed for 15 s, shaken at 250 rpm for 2 hours in the dark, and centrifuged (4500 rpm, 5 min) (Kerner *et al.*[176]).

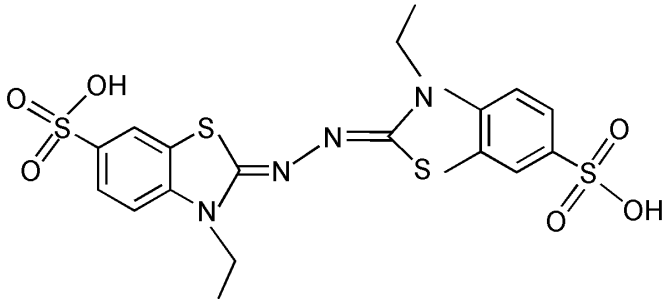

Fig. 2.3. $ABTS^{\bullet+}$ (2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid))

In Kerner *et al.*[177], $ABTS^{\bullet+}$ working solution by mixing 50 mL of ABTS (2 mM) with 200 μL of $K_2S_2O_8$ (70 mM) was prepared, and then 6 μL of the sample was added. The working solution was prepared by diluting with PBS (8.18 g NaCl, 0.27 g KH_2PO_4, 1.78 g $Na_2HPO_4 \times 2\ H_2O$, and 0.15 g KCl in 1 L of distilled H_2O). The sample was left in the dark for 15–16 h. The absorbance was measured at 734 nm, and the results were expressed as mg TE/g of extract (E) and dry pomace weight (dw).

2.4.3. Oxygen radical absorbance capacity (ORAC) assay

The oxygen radical absorbance capacity (ORAC) assay was performed as described by Prior *et al.*[178] and Dávalos *et al.*[179] by using fluorescein as a fluorescent probe. In short, 25 μL of the sample was mixed with 150 μL (14 μM) fluorescein solution and incubated for 15 min at 37 °C, followed by the addition of 25 μL of AAPH (240 mM) solution. The fluorescence was recorded in a *FLUOstar Omega Reader* (*BMG Labtech*, Offenburg, Germany); for every cycle (1 min x 1.1, in total, 120 cycles), we were using 485 excitation and 530 emission fluorescence filters. Antioxidant curves (fluorescence versus time) were first normalized, and, from the normalized curves, the net area under the fluorescein decay curve (AUC) was calculated: $AUC = (1 + f_1/f_0 + f_2/f_0 \ldots f_i/f_0) \times CT$, where f_0 is the initial fluorescence reading at 0 min, f_i is the fluorescence reading at time I, and CT is the cycle time in min. The final ORAC values were calculated by using a regression equation between Trolox concentration and the net AUC.

For BC ingredients, the reaction was carried out in 75 mM phosphate buffer (pH 7.4). Briefly, BC solids or cellulose (blank) were mixed with 150 μL of PBS solution (75 mmol/L) and 900 μL of fluorescein solution (14 μmol/L PBS), vortexed for 15 s, shaken at 250 rpm for 60 min in the dark, and centrifuged (4500 rpm, 5 min). 175 μL of supernatant was transferred to the 96-well black opaque microplates, pre-incubated for 15 min at 37 °C, followed by rapid addition of 25 μL of AAPH solution (240 mmol/L) as a peroxyl radical generator using a multichannel pipette. The fluorescence was recorded for every cycle (1 min x 1.1), with a total of 90–140 cycles. The results were expressed as μM TE/g dw.

2.4.4. DPPH• scavenging assay

DPPH• (2,2-diphenyl-1-picrylhydrazyl) (**Fig. 2.4**) scavenging capacity was measured with a slightly modified method of Brand-Williams *et al*.[180] by using a 96-well microplate reader *FLUOstar Omega Reader* (*BMG Labtech*, Offenburg, Germany). 7.5 µL of the extract was mixed with 300 µL DPPH• solution, and the decrease in absorbance was measured at 515 nm. The RSC values were calculated by using a regression equation: absorbance = 340.62 × Trolox conc. + 7.8965 (R2 = 0.99) produced with different concentrations of synthetic antioxidant Trolox. The results were expressed in milligrams of Trolox equivalent (TE)/g dw.

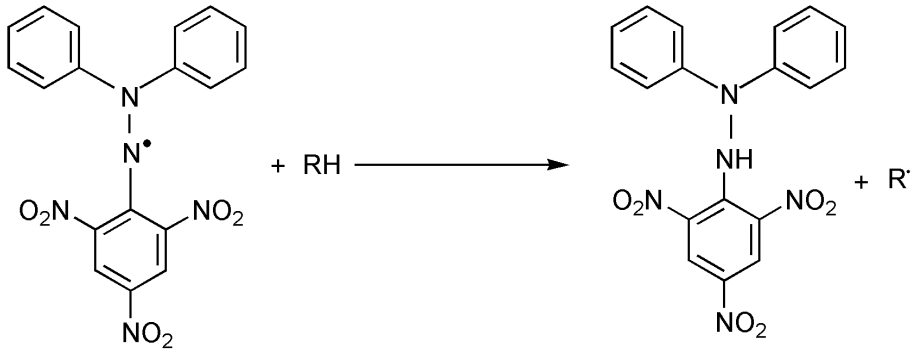

Fig. 2.4. DPPH• structure and its reduction by an antioxidant

2.5. Preparation of Pork Burger Patties, Pork Meatballs and Methods of their Analysis

The table with the recipes used in three research works is presented in **Tbl. 2.1.**

Table 2.1. Recipes used in three different researches

Sample	Ingredients (%)							
	Minced meat	**Water**	**Salt**	**SG**	**RH**	**DH**	**BC seeds**	**RB**
Experiment I								
Control	83.5	15	1.5	-	-	-	-	-
RH	81.5	15	1.5	-	2	-	-	-
DH	81.5	15	1.5	-	-	2	-	-
SG	83	15	1.5	0.5	-	-	-	-
RHSG	81.5	15	1.5	0.5	1.5	-	-	-
Experiment II								
Control	89	10	1	-	-	-	-	-
BC-RS1	88	10	1	-	-	-	1	-
BC-RS3	86	10	1	-	-	-	3	-
BC-RS5	84	10	1	-	-	-	5	-
BC-ASC1	88	10	1	-	-	-	1	-
BC-ASC3	86	10	1	-	-	-	3	-
BC-ASC5	84	10	1	-	-	-	5	-
BC-AE1	88	10	1	-	-	-	1	-
BC-AE3	86	10	1	-	-	-	3	-
BC-AE5	84	10	1	-	-	-	5	-
Experiment III								
Control	88	11	1	-	-	-	-	-
AC	86	11	1	-	-	-	-	2
E	87	11	1	-	-	-	-	1
R	86	11	1	-	-	-	-	2

RH – dried mechanically pressed hempseed cake; DH – defatted by supercritical CO_2 extraction hempseed cake; SG – sweet grass extract; RHSG – sweet grass extract and dried pressed hempseed cake; BC-seeds – blackcurrant seed ingredients at concentrations 1, 3 and 5%. BC-RS – blackcurrant seeds before CO_2 extraction; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction; BC-AE – blackcurrant seeds after EtOH/water extraction; AC – meatballs with defatted with supercritical CO_2 rowanberry pomace; E – meatballs with EtOH/water extract of AC; and R – meatballs with extraction residue; RB – rowanberry

2.5.1. Pork burger patties, packaging and storage

Minced pork meat was purchased from a local commercial abattoir (Tartu, Estonia). The mixture of patties was prepared with tap water and salt, and it was mixed manually until all of the ingredients were spread evenly. After mixing, the samples were divided into 5 portions: (1) control samples (83.5% of minced pork meat, 15% water, 1.5% salt); (2) samples with 2% dried hempseed press-cake flour (RH); (3) samples with 2% fully defatted hempseed press-cake flour (DH); (4) samples with 0.5% sweet grass extract (SG); and (5) samples with SG and RH, 0.5 and 1.5%, respectively (RHSG). Patties weighing 70 g (Ø 8.6 cm) were pressed from the raw mixture by using a hamburger press (*Indasia*, Greece). The patties were cooked in a preheated teflon-coated grill *Sage Smart Grill Pro Model BGR840 BSS* (*Breville*, Sydney, Australia) to an internal temperature of 75 °C measured with the temperature probe of the grill. Then, after cooling the patties down to room temperature, they were packed by using modified atmosphere consisting of 70% N_2 and 30% CO_2 (*Linde GAS AS*, Tallinn, Estonia) with a *Vision Pack Srl VP01* (*Packaging Factory Holding*, Lallio, Italy). All the samples were stored at 4 °C and analyzed after 0, 4, 8, 15, and 21 days of storage.

2.5.2. Pork meatballs, packaging and storage

Minced pork meat was purchased from a local commercial abattoir (Tartu, Estonia). The mixture of meatballs was prepared with tap water and salt, and it mixed manually until all of the ingredients were spread evenly. After mixing, the samples were divided into portions. Samples of meatballs with BC seed ingredients were as follows: the control sample (without BC ingredients) contained 89% of minced pork, 10% of tap water and 1% of salt; test samples contained 1%, 3%, and 5% of different BC seed ingredients (meat content 88, 86, and 84%, respectively). Meatballs with BC skin ingredients (part of the unpublished data): the control sample (without BC ingredients) contained 89% minced pork meat, 10% tap water, and 1% salt; meatballs with the addition of BC skin ingredients contained 87% minced pork, 10% tap water, 1% salt, and 2% BC skin ingredients. The mixture of meatballs with rowanberry pomace ingredients was divided into the following portions: the control sample (88% minced pork, 11% water, 1% salt), and the test samples with additives AC, R and E, each with the concentrations of 1%, 2%, 3%, and 5%. The raw mixture was shaped into meatballs with a weight of about 30 g, a diameter of 4.5 cm, and 2 cm of height by using a self-made form mould. The meatballs were cooked in a pre-heated oven *Inoxtrend E1 CUA-107E* (Treviso, Italy) at 145 °C × 15 min, and then cooled down to room temperature, packed under modified atmosphere conditions consisting of 70% N_2 and 30% CO_2 (*Linde GAS AS*, Tallinn, Estonia) with a *Vision Pack S.r.l. VP01* apparatus (*Packaging Factory Holding*, Lallio, Bergamo, Italy). All the samples with BC ingredients were stored at 4 °C and analysed at 1, 3, and 6 days of storage, with the exception of metabolomics, which was used to evaluate the optimum inclusion levels (%) by looking at specific marker compounds in the prepared meatballs. The packed meatballs with rowanberry pomace ingredients were stored at 4 °C and analyzed at 0 and 5 days of storage.

Three meatballs were used for analysis (pH, water activity a_w, and colour measurements) for each storage duration.

2.5.3. Determination of physico-chemical quality characteristics of pork burger patties and meatballs

Prior to chemical analyses, samples (at least 200 g) were ground and homogenized in a *Retsch GM200* laboratory homogenizer (*Retsch Gmbh & Co*, Haan, Germany). The grilled pork patties were analyzed for moisture (EVS-ISO 1442:1999), protein (EVS-ISO 937:1978, Kjeldahl method), fat (EVS-ISO 2446:2001, Gerber method), and ash content (ISO 936:1999). For pork meatballs with BC seed ingredients, also, the total dietary fibre was measured by using the *Megazyme Dietary Fibre Assay Kit* (K-TDFR-100A) (*Megazyme Ltd*, Wicklow, Republic of Ireland) by following the AOAC 991.43 method.

The grilling loss was measured after cooling the grilled pork burger patties to room temperature and by weighing the patties before and after the thermal treatment.

Cooking loss was measured after cooling the cooked pork meatballs to room temperature and by weighing the meatballs before and after thermal treatment. The grilling and cooking losses were expressed in percentages (%).

pH was measured with a *Seven 2Go*™ pH-meter (*Mettler-Toledo AG Analytical*, Schwerzenbach, Switzerland) by calibrating it before measurements with pH 4 and pH 7 buffer solutions. 5 g of the sample was homogenized with 50 mL of 0.1 M potassium chloride solution in *Retsch GM200* (ISO 2917:1999) laboratory homogenizer. Three replicate measurements were made.

Water activity (a_w) was measured in a water activity analyzer (*Aqua Lab*, Model Series 3 TE, *Decagon Devices, Inc.*, Washington, DC, USA) by placing the samples after opening the package into a closed measuring chamber of the water activity analyzer. Three measurements were done in total.

The colour was measured by using an *X-Rite 964* spectrophotometer (*X-Rite*, Grand Rapids, MI, USA). The colourimeter was calibrated before use on a black-and-white surface. After opening the package and cutting the samples into halves, the colour characteristics were measured from three different places of the samples to obtain the average value. The results were expressed by the CIE (International Commission on Illumination) Lab system values (D65 and the observer angle of 10°) with L^* indicating lightness, a^* indicating redness, and b^* for yellowness.

The total colour difference (ΔE_{Lab}) between the control and the test samples was calculated by the following formula (Equation 1) based on the three colour coordinates (CIE L^*, a^*, and b^*):

$$\Delta E_{Lab} = \sqrt{(L_0^* - L_1^*)^2 + (a_0^* - a_1^*)^2 + (b_0^* - b_1^*)^2}, \quad (1)$$

where ΔE_{Lab} is the total colour difference between the control and test samples; $L_0^*, a_0^*,$ and b_0^* are the means of the colour parameters determined for the

control samples; and $L_1^*, a_1^*, \text{and } b_1^*$ are the means of the colour parameters determined for the test samples.

Interpretation of the results:

- when $0 < \Delta E_{Lab} < 1$ – the observer does not notice the difference;
- when $1 < \Delta E_{Lab} < 2$ – only an experienced observer may notice the difference;
- when $2 < \Delta E_{Lab} < 3.5$ – an unexperienced observer also notices the difference;
- when $3.5 < \Delta E_{Lab} < 5$ – a clear difference in colour is noticeable, and;
- when $5 < \Delta E_{Lab}$ – an observer notices two different colours (Mokrzycki and Tatol[181]).

2.5.4. Measurement of thiobarbituric acid reactive substances (TBARS)

TBARS (**Fig. 2.5.**) was measured by using a method reported by Pikul *et al.*[182] with some modifications. 20 mL of 4% perchloric acid and 0.25 mL of butylated hydroxytoluene were homogenized with 5 g of the sample in an *Ultra-Turax IKA T18* homogenizer (*IKA*, Staufen, Germany), and filtered.

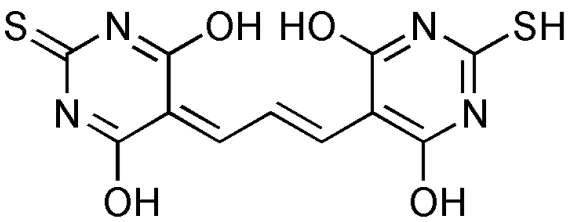

Fig. 2.5. Thiobarbituric acid reactive substances (TBARS)

The filtrate with TBA was heated in a water bath at 80 °C for 1 h and cooled. The absorbance was read at 538 nm, the values were expressed in malondialdehyde (MDA) mg/kg (**Fig. 2.6.**), while the changes were also expressed in MDA mg/kg by subtracting the measured value during storage from the value measured on day 0 (ΔMDA mg/kg).

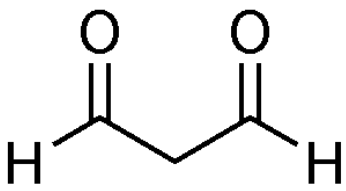

Fig. 2.6. Malondialdehyde (MDA)

2.5.5. Analysis of volatile aroma compounds

The volatile composition of the meat samples was analyzed by gas chromatography – time-off-flight mass spectrometry on a GC×GC-TOF/MS *LECO Pegasus* 4D system, consisting of an *Agilent 7890A GC*, a *GERSTEL Multipurpose Sampler MPS* (*Gerstel GmbH*, Mulheim an der Ruhr, Germany), coupled with a high-speed TOF/MS detector (*LECO*, St. Joseph, MI, USA). The volatile compounds of fresh minced meat and cooked meat balls samples were isolated by the headspace solid phase microextraction (HS-SPME) method according to Pavlidis *et al*.[183]. The sample preparation was as follows: 2.5 g of the meat sample and 5 mL of 25% (w/w) NaCl solution were added into a 20 mL glass vial (*Gerstel*) and homogenized with a glass rod for 2 min; then, the vial was closed with a magnetic cap with Silicone blue/PTFE white septa and vortexed at 1000 rpm for 15 min. HS-SPME was performed with an *MPS-2* autosampler (*Gerstel*, Mulheim an der Ruhr, Germany) with a Divinylbenzene/Carboxen/olydimethyl-siloxane fibre (50/30 μm, 1 cm in length, needle size 23 Ga, *Supelco*, Bellefonte, PA, USA). The vial was equilibrated at 40 °C for 15 min, and then, the fibre was exposed to the sample headspace for 30 min at 40 °C. Afterwards, the fibre was desorbed in the GC–TOF/MS injector for 180 s.

The column set consisted of a primary column *BPX-5* (30 m, 0.25 mm i.d., 0.25 μm film thickness) (*SGE Analytical Science*, Australia) connected to a secondary column, BPX-50 (1.2 m, 0.10 mm i.d., 0.1 μm film thickness). The primary oven was programmed as follows: 40 °C (1 min) ramped to 220 °C at 5 °C/min (1 min), finally ramped to 300 °C at 20 °C/min (hold 1 min), with a modulator offset temperature of +15 °C. The transfer line temperature was 270 °C, the GC injector port was set at 150 °C, and then, at a rate of 720 °C/min, ramped to 250 °C with a desorption time of 5 min. The carrier gas helium was set at 1 mL/min. The TOF/MS acquisition rate was 10 spectra/s, the mass range used for identification was from 30–450 m/z units. The detector's voltage was set at 1550 V, and the ion source temperature was set at 250 °C. Data from the GC × GC-TOF/MS system were collected by *ChromaTOF* software *v.4.22* (*LECO*) after a solvent peak delay of 250 s in a splitless mode for 60 s, a further valve was opened, and the purge flow was 20 mL/min; the mass spectrum assignment was based on matching against Adams, Nist, MainLib, RepLib mass spectral libraries; the signal-to-noise threshold was set as 50, with the minimum similarity accepted being 750. The mean values were calculated from quadruplicate injections.

The identification of volatile components was assigned by comparing their retention indices (RI) relative to C7–C30 standard n-alkanes, obtained on a nonpolar *BPX-5* column with the values provided in literature (Adams[184]), and by comparing their mass spectra with the data provided by the *Nist*, *MainLib*, *RepLib* and *Adams* mass spectral libraries. Positive identification was assumed when good match of the mass spectrum and RI had been achieved.

2.5.6. Sensory evaluation of pork burger patties and meatballs

The sensory evaluation of raw and grilled pork patties was conducted by eight randomly selected experienced assessors from the Estonian University of Life

Sciences, Chair of Food Science and Technology. The sensory analysis was carried out in a room with individual booths in a light-controlled room. Grilled patties were warmed to 60 °C in a microwave oven (*Moulinex Micro-Chef V98*, Écully, France), halved before the evaluation, and all the samples were presented on white dishes, labelled individually. The grilled patties were assessed on days 0, 4 and 8, and raw patties on days 0 and 8. The panellists were asked to evaluate the following descriptors for grilled patties: appearance, colour, odour, taste, texture, and juiciness; for the raw patties: appearance, colour, and odour grades were recorded. A hedonic 9-point scale was used for sensory evaluation as this approach has already been quite widely used for comparison purposes, particularly in cases involving the use of new ingredients.

The sensory evaluation of cooked meatballs with the addition of rowanberry ingredients was conducted by nine randomly selected trained assessors. The fresh meatballs were warmed to 55–70 °C in a microwave oven (*Moulinex Micro-Chef V98*, Écully, France) and cut in half before sensory assessment. The sensory attributes for the valuation of cooked meatballs were the odour, appearance, colour, taste, juiciness, and texture. A hedonic 9-point scale (9 – very good, 5 – satisfying, and 1 – not satisfying), as a widely used method (Wichchukit and O'Mahony[185]), was employed.

2.5.7. Untargeted metabolomics

Pork meatballs with BC seed ingredients were thawed at room temperature and then extracted following the protocol previously reported by Pateiro *et al*.[186], with minor modifications. Briefly, one gram of each sample was extracted with 10 mL of 80% aqueous methanol (v/v) solution (both LC-MS grade, *VWR*, Milan, Italy) added with 0.1% (v/v) formic acid. This mixture was subjected to an extraction system based on the utilization of an *Ultra-Turrax* (*Ika T10*, Staufen, Germany) for 5 min at room temperature. The corresponding extracts were centrifuged (*Eppendorf 5810R*, Hamburg, Germany) at 7.800 g for 15 min at 4 °C, and then filtered by using 0.22 μm cellulose syringe filters. Finally, the filtered samples were transferred to amber vials until instrumental analysis.

For the rowanberry extracts in meatballs, the untargeted metabolomics was used to evaluate the effect of the storage time and the phytochemical profile. The time points for metabolomics were day 0, as the day of preparation, day 4 and day 14 to assess the possible oxidation process of packed meatballs. For that, meatballs were lyophilized. Extraction was carried out as for meatballs with BC seed ingredients.

In the current experiment, untargeted profiling analysis was conducted by using *high-resolution mass spectrometry* (HRMS) based on a *Q-ExactiveTM Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer* (*Thermo Scientific*, Waltham, MA, USA) coupled to a Vanquish ultra-high-pressure liquid chromatography (UHPLC) pump and equipped with heated electrospray ionization (HESI)-II probe (*Thermo Scientific*, USA). Shortly, the chromatographic separation was carried out under a gradient of acetonitrile in water (from 6% to 94% in 35 min) as a mobile phase, with 0.1% formic acid as a phase modifier, by using BEH C18 (2.1x100 mm, 1.7 μm)

analytical column maintained at 35°C. The injection volume was 6 µL, and elution was operated with a flow rate of 200 µL/min. Full scan MS analysis was performed under the positive ionization mode and with a nominal mass resolution of 70,000 FWHM at m/z 200. The injection sequence was randomized, with three replicates for each sample. The quality control (QC) samples (prepared by pooling the same aliquots of each sample) were acquired in a data-dependent (TOP N = 3) MS/MS mode, and the Top N ions were selected for fragmentation under stepped (10, 20, 40 eV) Normalized Collisional Energy. The HESI parameters were previously optimized by Rocchetti *et al*.[187].

2.6. Statistical Analysis

Statistical analyses were performed with the statistical package *R 4* (R Core Team[188]). The effects of the variants, the storage period, and their interaction, and the random effect of batches (experimental replications) on the pH, colour characteristics, a_w, and TBARS of the amples were studied by the *Linear Mixed-Effects Model* (GLMM). The Emmeans (Lenth[189]) and multcomp (Hothorn *et al*.[190]) packages were used to carry out the pairwise comparison of the groups. Tukey's multiple comparison post hoc test was used to determine the groups' least square mean differences at the significance level of $\alpha = 0.05$. The effects of the variants and batches on the sample moisture, protein, and ash content as well as on the grilling and cooking loss were measured only on days 0 and 1, respectively, by GLMM. All model-assessed results are presented as least-square means. Boxplots charts were used to illustrate the results of the sensory evaluation by the *ggplot2* (Wickham[191]) package in *R 4.0.4*.

The multivariate statistical analyses to elaborate the metabolomics-based dataset were done by using two different types of software, namely, *Mass Profiler Professional* (*Version B.12.06*; from *Agilent Technologies*) and *SIMCA* (*Version 16*; from *Umetrics*, Malmo, Sweden) for data processing and normalization (Rocchetti *et al.*[187]). In this regard, both unsupervised and supervised multivariate statistics were used based on hierarchical cluster analysis (HCA), Principal Component Analysis (PCA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA).

3. RESULTS AND DISCUSSION

This chapter quotes passages from the articles with the permission of *Foods*[4] and *Elsevier*[5,6] journals.

3.1. Characterization of Plant-Based Ingredients

During the last decades, plant-based ingredients have become popular as possible substitutes of a more expensive ingredient – raw meat. In addition, this choice is related to the healthiness of meat products. Plant origin ingredients usually contain health-beneficial constituents, bioactive compounds, such as polyphenolic antioxidants, and dietary fibre. When using extracts, it is preferable to use green (environmentally and food-friendly) solvents and techniques, whereas the sensorial attributes of new plant ingredients should be acceptable for the food products. In the current study, green and novel extraction techniques were used for the removal of lipophilic components in order to obtain protein-enriched defatted hemp-seed cake as well as to obtain a strong antioxidant extract of sweet grass. To better understand the effects of plant-based ingredients used in meat products, selected chemical characteristics were observed.

In the first experiment, dried and mechanically pressed hempseed cake, defatted hempseed press-cake, and sweet grass extract was used in the production of pork patties. Hemp seeds contain a high amount of easily oxidizable unsaturated oil. This oil is a valuable product, and it is traditionally extracted by pressing. However, pressing recovers oil only partially; usually, press-cake still contains approx. 10% of oil, which may be extracted with a nonpolar solvent, *e.g.*, hexane, petrol ether. Considering that the use of flammable and toxic solvents is highly unfavourable for food applications, a green technology – SFE-CO_2 – was used. Moreover, a defatted press-cake contains a higher amount of proteins, which is important in the formulation of meat products. In fact, we tested these ingredients to compare their impact on the quality of meat products. The chemical composition of the hemp ingredients in use (as obtained from the company *Agropro* (Kaunas, Lithuania)) are listed in **Tbl. 3.1.** The protein content of the used hemp ingredients in meatballs were 36.6% in a dried mechanically pressed hempseed cake (RH, raw hemp) and 51.7% in defatted by scCO_2 hempseed cake (DH). According to the literature, the crude protein concentration in dehulled hemp seeds is between 30.3–38.7%, and, in hempseed meal, between 31–53.3% (House *et al.*[92]). The fibre content of DH was also slightly higher compared to RH. Defatted hemp had a weak odour, and its colour was noticeably lighter compared with RH.

[4] Application of Raw and Defatted by Supercritical CO_2 Hemp Seed Press-Cake and Sweet Grass Antioxidant Extract in Pork Burger Patties. https://doi.org/10.3390/foods10081904

[5] Evaluation of different blackcurrant seed ingredients in meatballs by using conventional quality assessment and untargeted metabolomics. https://doi.org/10.1016/j.meatsci.2023.109160

[6] Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs. https://doi.org/10.1016/j.fochx.2023.100761

Mechanically pressed hempseed press-cakes contain approximately 10% highly unsaturated residual oil. Therefore, the direct application to meat products is risky and may cause oxidation. From **Tbl. 3.1.**, we can see that, by defatting raw hempseed cake, we decreased significantly the fat content, and therefore the removal of residual oil should eliminate oxidation-related negative effects. Both used ingredients had a high fibre content. The findings of Kotecka-Majchrzak *et al*.[7] suggest that hemp cake may be used in food as a nutritious ingredient in the production of sustainable meat products.

No antioxidant activity of hempseed ingredients was detected, but, according to Pihlanto *et al*.[24] and Rea *at al*.[97], the TPC of dry defatted hempseed flour was between 5.88–10.63 mg (expressed in mg of gallic acid equivalents) per gram. Martinez *et al*.[96] isolated from the defatted hempseeds press-cake N-transcaffeoyltyramine as one of the main bioactives.

Table 3.1. Proximate chemical composition of hemp ingredients used in pork patties

Sample	Protein, %	Fat, %	Dietary fibre, %
RH	36.6	13.3	21.0
DH	51.7	1.4	26.1

*RH – dried mechanically pressed hempseed cake; DH – defatted by supercritical CO_2 extraction hempseed cake

In the preparation of sweet grass extract, the first extraction (SFE-CO_2) is used for removing lipids and volatile odour compounds (which may exert an impact on the sensory characteristics of a meat product), while the main – antioxidant-rich – product is obtained with polar solvent ethanol during the second extraction.

Sweet grass (SG) extract, as a previously characterized natural material, showed strong antioxidant activity (**Tbl. 3.2.**), especially in the ORAC assay (30.65 ± 1.64 mmol TE/g dw). Moreover, TPC observed in SG was 99.04 mg GA/g dw, $DPPH^{\bullet}$ 300.3 mg TE/g dw and $ABTS^{\bullet+}$ 692 mg TE/g dw). In a previous study, Bandonienė *et al*.[79] reported sweet grass extract as potential antioxidants in rapeseed oil, and 5,8-dihydroxycoumarin and its glycoside were recognized as the main radical scavengers. Bandonienė *et al*.[79] and Zainuddin *et al*.[83] tested SG in rapeseed oil and pork lard, where it performed with a strong antioxidant activity. It delivered the same effect as synthetic antioxidant BHT at the same concentration.

Table 3.2. Antioxidant activity of sweet grass extract

TPC (mg GA/g dw)	$DPPH^{\bullet}$		$ABTS^{\bullet+}$		ORAC, (mmol TE/g dw)
	(mg TE/g dw)	(IC_{50})	(mg TE/g dw)	(IC_{50})	
99.04 ± 1.61	300.2 ± 1.7	0.02	692 ± 8.2	0.09	30.65 ± 1.64

*TPC – total phenolic content; $DPPH^{\bullet}$ – 2,2-diphenyl-1-picrylhydrazyl hydrate free radical; $ABTS^{\bullet+}$ – 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid; ORAC – oxygen radical absorbance capacity

In the second experiment, BC seed ingredients were used. The seeds and skins were from the same blackcurrant pomace. The proximate chemical composition of BC seed ingredients used is presented in **Tbl. 3.3.**

Table 3.3. Proximate chemical composition of BC seed residues after extractions. Values are the least square means ± standard error.

Sample	Moisture, %	Protein, %	Minerals, %
BC-RS	5.83 ± 0.14	20.17 ± 0.04	3.24 ± 0.03
BC-ASC	7.86 ± 0.20	21.15 ± 0.33	3.68 ± 0.05
BC-AE	7.45 ± 0.15	22.53 ± 0.31	2.44± 0.005

*BC-RS – blackcurrant seeds before CO_2 extraction; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction; BC-AE – blackcurrant seeds after EtOH/water extraction

Both the environment and genetics play a critical role in the production, chemistry, and nutritional quality of BC fruits. The composition of BC fruits strongly depends on the berry cultivar and environment. There are no spectacular differences in the proximate composition of BC seed extracts; however, there is some slight increase in the protein content of the extraction residues. It could be explained by the elimination of compounds soluble in supercritical CO_2, EtOH and water during each extraction stage, which results in somehow higher proportions of insoluble proteins in the residues. The currently available information allows concluding that extraction residues have a higher content of proteins than meat. According to Alba *et al.*[192], blackcurrant pomace contains insoluble dietary fibres at around 47% and soluble fibres at a level of ∼28%.

The study carried out by Lu and Foo[193] highlighted that blackcurrant seed residues after oil extraction had a similar phenolic composition to that found in the berries. The *in vitro* antioxidant values measured in the current study for BC seed ingredients are presented in **Tbl. 3.4.** In comparison with the raw dried seeds, all the values in the $scCO_2$ extraction residue were significantly higher. It can be explained by the removal of lipophilic compounds and oil, which led to the increased proportion of polyphenolic antioxidants and an improved availability of antioxidative active groups for the electron/hydrogen atom transfer in the determination reaction system. Lu and Foo[193] reported that the phenolic composition of BC seed residue after the extraction of lipophilic compounds with $scCO_2$ was similar to that of the whole berries. All the values decreased after an additional re-extraction of the residue by using the polar solvents EtOH and water, whereas TPC and $ABTS^{\bullet+}$ values remained high. Some differences in ORAC can be explained by the differences in the assays; TPC and $ABTS^{\bullet+}$ are based on SET (single electron transfer), while ORAC measures the ability to inactivate peroxy-radicals by donating hydrogen. It may be assumed that EtOH/water extraction efficiently removed hydrogen donating polyphenolic antioxidants, while the antioxidants and/or the functional groups participating in the SET mechanisms may be both extracted and formed or become available during and after extraction, respectively. Jurčaga *et al.*[194] compared the scavenging of Kamchatka honeysuckle (*Lonicera caerulea* var. Kamtschatica) extract and extract of blackcurrant berries and found the

latter to be less effective. The BC extract also had a lower anthocyanin content (2588.37 and 4809.43 mg L^{-1}) and TPC compared to Kamchatka honeysuckle (16.32 and 38.67 g GAE kg^{-1}, respectively).

Table 3.4. Total phenolic content and *in vitro* antioxidant activity of blackcurrant seeds and their residues after extractions.

Sample	TPC, mg GA/g	$ABTS^{\bullet+}$, TE mg Trolox/g	ORAC, TE mg Trolox/g
BC-RS	42.07 ± 0.53	74.12 ± 0.99	13.35 ± 0.52
BC-ASC	62.09 ± 1.16	141.31 ± 3.00	15.62 ± 0.46
BC-AE	31.54 ± 0.87	109.63 ± 1.60	2.73 ± 0.05

*BC-RS – blackcurrant seeds before CO_2 extraction; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction; BC-AE – blackcurrant seeds after EtOH/water extraction

The *in vitro* antioxidant values measured (part of unpublished data) for BC skin ingredients are presented in **Tbl. 3.5.**

Table 3.5. Total phenolic content and *in vitro* antioxidant activity of raw blackcurrant skin, its extraction residues, and extract.

Sample	TPC, mg GA/g	$ABTS^{\bullet+}$, TE mg Trolox/g	ORAC, TE mg Trolox/g
R	38.24 ± 0.47	95.4 ± 3.2	62.91 ± 0.14
AC	41.17 ± 0.23	98.4 ± 6.9	75.22 ± 0.67
HOE	125.6 ± 3.2	658.2 ± 10.9	701.7 ± 6.2
AA	9.36 ± 0.31	22.6 ± 1.8	18.14 ± 0.54

*R – raw blackcurrant pomace skins; AC – blackcurrant pomace skins after CO_2 extraction; HOE – combined extract of defatted residues consecutively isolated with EtOH and H_2O; AA – blackcurrant pomace skins after all extractions

In terms of the antioxidant activity (Sarv *et al.*[111]), three rowanberry pomace powders of cultivars Likernaja, Solnechnaja, and wild rowanberry were selected to be used in pork meatballs. Likernaja presented the highest $DPPH^{\bullet}$ value (527.55 μM TE/g dw), whereas Solnechnaja was denoted by an exceptionally high ORAC value (146.6 μM TE/g dw) (**Tbl. 3.6.**). The highest antioxidant activity of cultivar Likernaja among other hybrids was also reported by Jurikova *et al.*[195] and Kampuss *et al.*[196], who found the TPC values to be the highest for Likernaja (484.9 mg/100 g dw). Already Hukkanen *et al.*[113] found fruits of *S. aucuparia* to have high scavenging of $DPPH^{\bullet}$ and lipid peroxidation inhibition compared to other species.

Table 3.6. Antioxidative characteristics of rowanberry pomace (Sarv *et al.*[111]).

	TPC, mg GA/g dw	DPPH$^{\bullet}$ µM TE/g dw	ABTS$^{\bullet+}$ µM TE/g dw	ORAC µM TE/g dw
Likernaja	41.3	527.6	508.9	128.5
Solnechnaja	28.3	324.5	321.9	146.6
Wild rowanberry	31.7	358.6	313.2	135.2

*TPC – total phenolic content; DPPH$^{\bullet}$ – 2,2-diphenyl-1-picrylhydrazyl hydrate free radical; ABTS$^{\bullet+}$ – 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid; ORAC – oxygen radical absorbance capacity

All those three pomace powders were mixed, ground and defatted by supercritical CO_2 to obtain the first ingredient (AC). The second ingredient was EtOH/water microwave extract of defatted pomace (E), and the third ingredient was the extraction residue (R). According to the preliminary TPC analyses of those 3 ingredients, the TPC value of ingredient E was almost five-fold compared to the TPC value of AC and 17 times higher than that of R (**Fig. 3.1.**).

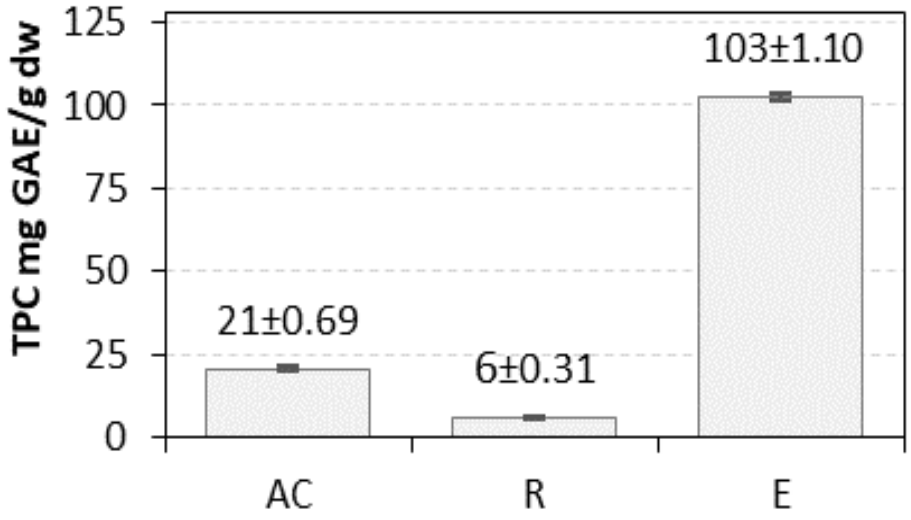

Fig. 3.1. Total phenolic content (TPC) of rowanberry pomace ingredients. AC – defatted with supercritical CO_2 rowanberry pomace; E – EtOH/water extract of AC; R – extraction residue

3.2. Effect of Plant-Based Ingredients on the Physico-Chemical Parameters of Pork Meat Products

3.2.1. Effect on proximate composition and cooking losses

As expected, the addition of a small amount of plant origin ingredients to grilled pork patties did not have any noteworthy effect on the proximate chemical composition (**Tbl. 3.7.**). However, a significantly lower moisture content was in RH and SG, which, in terms of hemp ingredients, corresponds to the data provided by Zajac *et al*.[22] who noted a lower moisture content in all the meat loaves with hemp additives compared to the control sample. Sun *et al*.[197] incorporated hempseed meal, which is a by-product from hempseed oil extraction, into chicken sausage (10, 20, 30, and 40%), and, from their results, it turned out that the moisture, protein and fat content decreased in a dose-dependent manner, but the ash and total dietary fibre contents increased ($p<0.05$).

Table 3.7. Proximate composition of grilled pork patties and grilling losses. Values are the least square means ± standard deviation.

Sample	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Grilling loss (%)
Control	61.68 ± 4.94^{a}	19.83 ± 1.61^{a}	16.84 ± 6.53^{ab}	2.48 ± 0.24^{a}	24.20 ± 8.18^{ab}
DH	63.36 ± 5.11^{a}	18.84 ± 1.77^{a}	15.85 ± 4.76^{a}	2.59 ± 0.30^{a}	14.34 ± 3.89^{c}
RH	59.12 ± 4.30^{b}	19.68 ± 1.29^{a}	16.80 ± 5.80^{ab}	2.59 ± 0.16^{a}	20.89 ± 4.21^{ad}
RHSG	58.82 ± 3.05^{b}	18.82 ± 0.92^{a}	16.68 ± 5.18^{a}	2.45 ± 0.16^{a}	19.45 ± 6.84^{d}
SG	57.75 ± 2.94^{b}	19.32 ± 0.86^{a}	18.91 ± 4.20^{b}	2.58 ± 0.25^{a}	26.21 ± 5.91^{b}

$^{a, b, c, d}$ – different letters in columns indicate significant differences between means ($p<0.05$) by Tukey's multiple comparison's post-hoc test. Control – without plant-based ingredients; RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract and 1.5% dried pressed hempseed cake; SG – 0.5% sweet grass extract

Despite the fact that cooking can improve meat tenderness, heat treatment also causes a weight loss. The grilling loss affects the juiciness of meat products, which is linked to consumer preferences. Due to the compositional complexity of meat products together with the impacts of grilling, it is fairly challenging to explain the indicated differences. It should be also highlighted that the SDs were rather high for the measured characteristics, and it may be explained by the heterogeneous biomaterials used in the experiments. The higher fat content in SG may be explained by the higher grilling losses and a lower moisture in the SG samples causing the proportional increase in the fat content. From the current research, it can be seen that the lowest grilling loss was observed in DH (14.34%) compared to the control sample (24.2%), explained by the high protein content (51.7%) in this ingredient, and therefore having a good water-holding capacity (WHC). WHC describes the ability of proteins to retain water. It was also reported by Raikos *et al*. (2014) that hemp flour had a stronger WHC than flours prepared from fava bean, buckwheat, or green pea due to its high protein content. It may be also hypothesized as being

related with the presence of edestin (which is a major constituent of the hempseed protein), which is generously present in hemp. Edestin is a globulin protein, which consists of about 75% of total protein. The same tendency was observed in samples RH (protein content 36.6%) and RHSG. The water-holding capacity of hemp may be a reflection of their high protein content, as also stated by Raikos *et al.*[198] and Xu *et al.*[90]. The same conclusion was provided by Zajac *et al.*[22] when 5% of hempseed ingredients in pork loaves reduced the cooking loss. This knowledge may be useful for the producers, as proteins are essential components in food processes helping to improve, for instance, the water-binding capacity, and therefore reduce production losses.

From **Tbl. 3.8.**, it is evident that the addition of BC seed ingredients affected the chemical composition and the cooking loss of pork meatballs. Almost all the samples had a higher protein content compared to the control sample, while the fat content decreased.

Table 3.8. Proximate chemical composition and cooking losses of pork meatballs. Values are the least square means ± standard error.

Sample	Moisture (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	Fibre content (%)	Cooking loss (%)
Control	53.27 ± 1.79^{ab}	21.32 ± 0.47^{bc}	18.98 ± 0.60^{c}	2.06 ± 0.046^{a}	0.00 ± 0.00^{f}	26.79 ± 1.59^{a}
BC-RS1	53.82 ± 2.95^{ab}	21.77 ± 0.57^{ab}	18.73 ± 1.09^{bc}	2.13 ± 0.011^{a}	0.181 ± 0.004^{a}	27.30 ± 1.31^{a}
BC-RS3	53.80 ± 3.64^{ab}	21.71 ± 0.19^{abc}	16.73 ± 1.29^{abc}	2.03 ± 0.030^{a}	1.104 ± 0.028^{b}	23.96 ± 0.81^{ab}
BC-RS5	54.57 ± 2.12^{a}	20.29 ± 0.57^{c}	17.17 ± 0.43^{abc}	1.96 ± 0.019^{a}	1.608 ± 0.020^{c}	20.48 ± 2.22^{b}
BC-ASC1	51.67 ± 3.67^{b}	22.88 ± 0.41^{a}	15.97 ± 1.21^{ab}	2.00 ± 0.022^{a}	0.206 ± 0.009^{a}	25.23 ± 1.74^{ab}
BC-ASC3	54.80 ± 4.05^{a}	21.87 ± 0.46^{ab}	15.06 ± 1.45^{ad}	1.94 ± 0.086^{a}	1.327 ± 0.016^{d}	22.82 ± 1.83^{ab}
BC-ASC5	52.75 ± 0.03^{ab}	22.85 ± 0.75^{a}	12.54 ± 0.98^{d}	1.98 ± 0.007^{a}	2.128 ± 0.016^{e}	19.77 ± 2.18^{b}
BC-AE1	53.47 ± 3.91^{ab}	22.32 ± 0.6^{ab}	16.88 ± 1.12^{abc}	2.05 ± 0.053^{a}	0.191 ± 0.004^{a}	27.05 ± 1.86^{a}
BC-AE3	54.71 ± 2.56^{a}	21.85 ± 0.35^{ab}	16.36 ± 0.67^{abc}	1.92 ± 0.087^{a}	1.074 ± 0.047^{b}	24.45 ± 1.56^{ab}
BC-AE5	55.75 ± 2.91^{a}	21.56 ± 0.46^{abc}	14.54 ± 1.23^{ad}	1.48 ± 0.128^{b}	1.585 ± 0.034^{c}	20.64 ± 1.10^{b}

$^{a, b, c, d}$ – Different letters in columns indicate significant differences between means (P<0.05) by Tukey's multiple comparison's post hoc test. Control – without plant-based ingredients; BC-RS – blackcurrant seeds before CO_2 extraction at concentrations 1, 3 and 5%; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction at concentrations 1, 3 and 5%; BC-AE – blackcurrant seeds after EtOH/water extraction at concentrations 1, 3 and 5%

Meat and meat products are lacking of fibre; therefore, the importance of fibres in meat products is very high. Fibres help to influence the physico-chemical characteristics of meat products and increase their nutritional and functional value. As fibres are present in fruit skins and seeds, they can be used in meat products also due to their technological properties. Blackcurrant pomace is a good source of soluble (~28%) and insoluble dietary (~47%) fibres (Alba *et al.*[192]). Based on the current data, the higher is the inclusion level of BC seed ingredients, the higher is the fibre content in pork meatballs, which confirms the hypothesis that, after CO_2 extraction, the percentage of dietary fibre increases as the non-polar compounds, such as oil, are removed during the extraction – that is, the amount of the starting material decreases, but the amount of fibre remains the same. Compared to other samples, the highest dietary fibre content was observed in samples BC-ASC.

The cooking loss is of great economic importance to the meat industry, which occurs by evaporation of moisture and loss of fat. From the sensory point of view, a high cooking loss can influence the eating quality. In all meatballs with the addition of 5% of BC seed ingredients, the cooking loss was lower compared to the control and other samples with lower concentrations. Meatballs with BC-ASC5 showed the lowest cooking loss, while having a high protein and fibre content (22.85 g/100g and 2.128%, respectively). It may be attributed to the high water-holding capacity and the ability of plant-based fibres to bind water. This finding is in agreement with Fang *et al.*[43], when the addition of 3% of sugarcane fibre increased the cooking yield, or with Choi *et al.*[199], who added *Laminaria japonica* powder (1, 3 and 5%) to pork patties and reported an increase in the fibre content and a decrease in the cooking loss. The highest cooking loss was observed in meatballs with BC-RS1 (27.30%), followed by BC-AE1 (27.05%) explained by the higher concentration of BC seed ingredients, which resulted in a lower cooking loss.

In the next experiment, as it was expected in the first article "Application of raw and defatted by supercritical CO_2 hemp seed press-cake and sweet grass antioxidant extract in pork burger patties", the same tendency was observed that the addition of a small amount (1–2%) of rowanberry pomace ingredients did not have any significant effect on the chemical composition of pork meatballs (**Tbl. 3.9.**). However, the fat content in meatballs decreased, especially in meatballs with fibre-rich ingredient AC and R. The same effect was detected in the previous study, when, with the addition of fibre-rich ingredients, the fat content slightly decreased. Extraction residue R had a higher moisture content, which therefore affected the juiciness, compared to the control sample, and also resulted in the lowest cooking loss (20.17%). It is in agreement with Mena *et al.*[200], who stated that, by-adding 3% of sugarcane fibre into meatballs, the cooking loss decreased. On the other-hand, lyophilized EtOH extract E increased the cooking loss significantly as it does not contain any fibres, but only EtOH-extracted soluble compounds (polyphenols).

Table 3.9. Proximate composition of cooked pork meatballs and cooking losses.

Sample	Moisture (g/100g)	Protein (g/100g)	Fat (g/100g)	Ash (g/100g)	Cooking loss (%)
Control	57.90±2.33[a]	20.74±0.35[a]	21.05±0.86[b]	2.04±0.276[a]	23.33±2.05[ab]
AC (2%)	58.48±4.03[a]	20.59±0.24[a]	15.20±4.54[a]	2.00±0.045[a]	24.27±2.42[ab]
E (1%)	57.62±2.13[a]	20.64±1.22[a]	19.33±1.38[bc]	1.85±0.027[a]	26.23±4.97[a]
R (2%)	59.31±1.83[a]	19.40±1.25[b]	17.00±0.30[ac]	1.94±0.109[a]	20.17±3.66[b]

[a, b, c] Different letters in columns indicate significant differences between the least square means ($p<0.05$) by Tukey's multiple comparison's post hoc test. Control – meatballs without plant-based ingredients; AC (2%) – meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace; E – meatballs with 1% of EtOH/water extract of AC; R – meatballs with 2% of extraction residue

3.2.2. Colour changes during storage

The colour of raw and cooked meat is one of the most important quality parameters, especially in terms of consumer purchasing preferences (Font-i-Furnols and Guerrero[201]), which is also in correlation with the overall meat quality. The meat colour is determined by two proteins: myoglobin (in the muscle) and haemoglobin (in the blood). In addition to the storage temperature, pH, and packaging, different plant-based ingredients have an effect on the colour of raw and cooked meatballs, therefore influencing the acceptance and appearance of meat. Typically, compounds produced via different oxidations (lipid, protein, pigment) are the cause of discolouration of meat and meat products. Various berry pomace extracts inhibit oxidative reactions, and therefore they may protect meat products from discolouration during storage. Jia *et al*.[62] suggested that blackcurrant extract inhibits the discolouration of meat products during cold storage.

The impact on colour characteristics and changes during storage of meat products (**Tbl. 3.10.** and **Tbl. 3.11.**) were evaluated by the CIE (International Commission on Illumination) Lab system, where L^* indicated lightness, a^* represented redness, and b^* stood for yellowness. All of the used ingredients affected the colour characteristics of pork meat products.

All of the ingredients used are green in colour, specifically, SG as dark green, RH lighter green, and the lightest ingredient was DH, explained by the presence of chlorophylls. The green colour generally is not acceptable for the consumers, especially when its origin is unknown. The lightness of DH can be explained by the extraction with $scCO_2$, which removes chlorophylls with residual lipids. Obviously, all the ingredients decreased the L^* value significantly; remarkably, sweet grass extract produced better results than that of DH and RH.

Table 3.10. Changes of colour parameters of pork patties during the storage period (days). Values are the least square means ± standard deviation.

Sample	Storage period (days)				
	0	4	8	15	21
	Lightness *L**				
Control	71.86±7.89^{Ca}	72.65±8.16^{Da}	72.79±8.16^{Da}	75.03±6.39^{Ca}	72.25±7.82^{Da}
DH	68.70±9.19^{BCa}	67.77±11.66^{BCa}	66.76±11.01^{BCa}	69.90±8.91^{Ba}	70.03±8.03^{CDa}
RH	67.70±7.42^{Ba}	69.20±8.13^{CDa}	68.10±9.56^{Ca}	69.26±7.10^{Ba}	67.60±6.11^{BCa}
RHSG	57.38±8.81^{Aa}	61.74±6.36^{Abc}	62.08±7.94^{Ac}	61.48±6.79^{Abc}	57.89±8.67^{Aab}
SG	60.50±9.73^{Aa}	64.39±8.42^{ABb}	63.67±7.74^{ABab}	64.63±5.96^{Ab}	64.18±7.85^{Bab}
	Redness *a**				
Control	7.67±5.45^{Aa}	7.08±4.38^{ABa}	7.01±4.45^{ABa}	6.73±4.59^{ABa}	6.81±4.54^{Aa}
DH	7.62±5.92^{Aa}	7.57±4.88^{Ba}	8.01±5.89^{Ba}	7.83±5.80^{Ba}	7.29±5.65^{ABa}
RH	7.73±6.02^{Aa}	7.59±6.29^{Ba}	7.48±6.04^{ABa}	7.89±6.29^{Ba}	8.71±6.49^{Ba}
RHSG	6.55±5.99^{Aa}	6.07±6.78^{ABa}	6.28±6.72^{Aa}	6.41±6.30^{ABa}	6.33±5.74^{Aa}
SG	6.24±6.45^{Aa}	5.81±6.29^{Aa}	6.13±6.56^{Aa}	6.15±6.80^{Aa}	6.20±6.08^{Aa}
	Yellowness *b**				
Control	28.06±15.18^{Aa}	27.51±15.34^{Aa}	28.07±15.24^{Aa}	26.32±12.92^{Aa}	26.54±13.29^{Aa}
DH	28.40±15.00^{Aa}	28.48±13.92^{Aa}	28.86±14.14^{Aa}	27.86±12.26^{ABa}	25.93±11.91^{Aa}
RH	30.26±14.05^{Aa}	29.29±14.88^{Aa}	29.73±14.92^{Aa}	27.72±12.46^{ABa}	26.74±12.40^{Aa}
RHSG	36.06±14.07^{Bc}	33.13±14.43^{Babc}	34.30±14.57^{Bbc}	31.05±11.46^{BCab}	29.50±10.09^{ABa}
SG	35.64±14.23^{Bb}	33.47±14.65^{Bab}	34.45±15.32^{Bab}	32.23±11.71^{Ca}	32.26±11.47^{Bab}

The least square means followed by the different capital letters in the columns and lower-case letters in the rows differ significantly by the Tukey's multiple comparison's post-hoc test (p<0.05). Control – without plant-based ingredients; RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract; 1.5% dried pressed hempseed cake; SG – 0.5% sweet grass extract

Although, it is interesting that there was some increase in the *L** values during the storage in samples with SG, most likely, due to the degradation of chlorophylls added with SG and the effects of antioxidants on meat pigments.

The decrease in lightness with hemp ingredients is in agreement with the findings of Zajac *et al.*[22], who reported a control sample of pork loaves to be lighter compared to those with hemp flour and hemp protein, and on yellowness values increasing; in the current study, it was particularly prominent in pork patties with RHSG and SG. Leonard *et al.*[202] incorporated lupin flour into beef sausage and noticed the trend of decreasing *L** values, along with increasing both *a** and *b** values in cooked beef sausage. Carvalho *et al.*[203] prepared chicken burgers by replacing meat with spinach (*Spinacia oleracea* L.). As expected, all the colour parameters were affected (p<0.01) by the addition of a dark green leafy vegetable; lightness was especially affected, which decreased. *a** as an important colour attribute of meat remaining stable during the storage period.

The changes in *L**, *a** and *b** values of pork meatballs with BC seed ingredients during chilled storage are presented in **Tbl. 3.11.**, from where significant differences can be observed due to the intensive colour of the added ingredients. The

lightest sample during the 6-day storage was the control sample. According to Schmidt *et al.*[204], the *L** value is an important parameter indicating whether a food product is dark or light. Similar results were obtained by Jurčaga *et al.*[194] by adding blackcurrant and Kamchatka honeysuckle extracts in frankfurters. It was obvious that the higher is the concentration of BC seed ingredients, the darker is the sample (a lower *L**-value). Also, Jia *et al.*[62] reported the decrease in lightness in pork patties with the addition of blackcurrant extract during 9 days of storage; Anton *et al.*[71] noticed the reduction of lightness in raw and cooked minced meat with blackcurrant powder by giving it a dark purple colour.

Parameter *a** (redness) significantly decreased in the control sample (from 10.14 to 7.77) during the 6-day storage period. The progressive increase of *a** was observed in all the samples with BC seed ingredients compared to the control sample; on the other hand, after 6 days of storage, the redness values decreased in all the samples. High *a** values indicate a redder colour, while the low redness values refer to a greener colour (Schmidt *et al.*[204]). The redness of meatballs with blackcurrant seeds after EtOH/water extraction was more intense than that of the control sample on the first day of storage (13.27 vs. 10.14, respectively). The increase in redness can be explained by the high content of anthocyanin pigments in BC, which has a remarkable effect on the colour of pork meatballs (**Fig. 3.2.**). Anthocyanins are known as natural water-soluble pigments responsible for the colour of blackcurrant berries. Anthocyanins cyanidin and delphinidin are typical in blackcurrant fruits (Šimerdová *et al.*[205]). The pigment stability is related to the pH value of the media. The colour of anthocyanins is more stable in acidic conditions (it is most stable at pH<3); therefore, an increase in pH could affect the decomposition of pigments and cause the decrease in colour of the meat samples.

Fig. 3.2. Meatballs with the addition of BC seed ingredients compared to the control sample

Table 3.11. Effect of BC seed ingredients and storage period (days) on the colour characteristics (*L**, *a** and *b**) of cooked pork meatballs.

Sample	Storage period (days)		
	1	3	6
	Lightness *L**		
Control	81.45±1.64^{fA}	81.83±1.59^{eA}	83.17±0.79^{gA}
BC-RS1	77.25±2.82^{afA}	78.95±1.26^{beA}	80.08±2.01^{agA}
BC-RS3	71.44±3.25^{bcA}	72.72±2.34^{acA}	73.1±3.07^{bcA}
BC-RS5	65.98±2.73^{eA}	66.11±2.36^{dA}	67.99±2.87^{dfA}
BC-ASC1	76.13±2.78^{aA}	77.99±1.52^{beA}	77.82±1.90^{aA}
BC-ASC3	67.93±3.53^{ceA}	71.98±2.79^{cB}	72.92±2.69^{cB}
BC-ASC5	60.06±1.95^{dA}	61.63±1.59^{dA}	63.48±2.81^{efA}
BC-AE1	74.94±3.21^{abA}	76.61±0.94^{abA}	77.26±1.4^{abA}
BC-AE3	70.58±4.93^{cA}	73.33±3.47^{acA}	71.45±5.31^{cdA}
BC-AE5	60.55±2.97^{dA}	63.83±4.91^{dB}	63.04±2.59^{eAB}
	Redness *a**		
Control	10.14±2.22^{cA}	9.25±1.62^{cA}	7.77±1.13^{cB}
BC-RS1	12.05±1.39^{abA}	11.38±0.83^{abAB}	10.26±1.88^{bB}
BC-RS3	11.61±1.51^{abcA}	11.82±0.50^{abA}	11.67±0.77^{abA}
BC-RS5	11.69±0.65^{abcA}	11.28±0.57^{abA}	10.98±0.45^{abA}
BC-ASC1	12.61±1.21^{aA}	11.97±1.38^{abA}	11.47±1.24^{abA}
BC-ASC3	12.44±1.4^{abA}	10.9±1.09^{bcB}	11.49±0.83^{abAB}
BC-ASC5	11.52±0.59^{abcA}	11.17±0.66^{abA}	10.44±0.75^{abA}
BC-AE1	13.27±1.32^{aA}	12.93±1.04^{aA}	12.1±1.12^{aA}
BC-AE3	12.31±0.74^{abA}	11.58±1.01^{abA}	11.57±0.98^{abA}
BC-AE5	10.88±0.55^{bcA}	10.36±1.27^{bcA}	10.54±0.51^{abA}
Sample	Storage period (days)		
	1	3	6
	Yellowness *b**		
Control	41.18±1.19^{eA}	40.24±0.92^{aA}	40.18±1.47^{fA}
BC-RS1	38.60±1.36^{aeA}	38.24±1.14^{aA}	38.70±0.87^{bfA}
BC-RS3	33.86±3.19^{cA}	33.97±0.99^{bA}	34.56±0.74^{acA}
BC-RS5	31.92±1.79^{cA}	31.84±1.08^{bA}	31.75±1.07^{dA}
BC-ASC1	36.89±3.08^{abA}	38.35±1.21^{aA}	37.75±1.39^{bfA}
BC-ASC3	32.72±1.44^{cA}	32.64±1.02^{bA}	33.13±1.42^{cdA}
BC-ASC5	28.54±0.99^{dA}	28.85±1.15^{cA}	28.69±0.97^{eA}
BC-AE1	37.79±0.71^{aA}	37.96±1.23^{aA}	36.36±4.30^{abA}
BC-AE3	34.29±2.81^{bcA}	34.39±1.86^{bA}	33.77±3.42^{acdA}
BC-AE5	28.63±1.17^{dA}	28.31±0.69^{cA}	28.62±1.42^{eA}

*Control – without plant-based ingredients; BC-RS – blackcurrant seeds before CO_2 extraction at concentrations 1, 3 and 5%; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction at concentrations 1, 3 and 5%; BC-AE – blackcurrant seeds after EtOH/water extraction at concentrations 1, 3 and 5%

The same tendency for *L** and *a** values was observed by Ganhão *et al.*[17], Jia *et al.*[62], Chung *et al.*[206], and Tamkutė *et al.*[171], who used different berries, including

blackcurrant, in meat products. De Santos *et al.*[207] highlights that redness values ranging from 4.6 to 10.8 are perceived as brown in pork; therefore, meatballs with BC-AE5 and the control sample look brown during the 6 days of storage. Since the control sample underwent heat treatment, it shows an expected trend.

Parameter *b** (yellowness) was the highest in the control samples and remained the same during the storage period. It was noticed that the higher is the concentration of BC seed ingredients, the lower are the *b** values. The lowest yellowness values were in meatballs with 5% of BC seeds after EtOH/water extraction and 5% of seeds after supercritical fluid CO_2 extraction. According to Esmer *et al.*[208], a decrease in the *a** value, as the loss of redness and the formulation of metmyoglobin, leads to a decrease in the *b** value. Researchers distinguished discolouration in products with plant-based ingredients, which is explained by the reactions between amines and oxidation products in meat (Peiretti *et al.*[209]).

In the next experiment, rowanberry pomace ingredients were used. The appearance of meatballs with the addition of rowanberry pomace EtOH/water extract is presented in **Fig. 3.3** showing the characteristic colour and the increased redness.

Fig. 3.3. Meatballs with the addition of rowanberry pomace EtOH/H_2O extract

The *L** value of meatballs with rowanberry pomace ingredients decreased (**Fig. 3.4a**), with a great probability due to anthocyanins in rowanberries. The same effect was seen with the addition of dark-coloured blackcurrant seed ingredients in pork meatballs. A similar tendency was also noticed by Peiretti *et al.*[209] by adding blueberry pomace to chicken patties, or by Selani *et al.*[210] with the addition of grapes and blackcurrants into the raw and cooked chicken meat. Its redness (*a**) increased up to 48% (**Fig. 3.4b**). In the control sample, the redness values were 10.38 on the preparation day, yet they decreased to 8.77 after 5 days of storage. Those values are between 4.6 to 10.8, which is perceived as brown in pork according to De Santos *et al.*[207], and which shows an expected trend for cooked meat products. Rowanberry pomace extracts led to some decrease of yellowness (*b**) (**Fig. 3.4c**), especially with the addition of high bioactive components 1%-E and 2%-AC by 1.87% and 0.42%, respectively, after cooking, as detected again by Peiretti *et al.*[209]. On the other hand, ingredient 2%-R increased the yellowness by 0.51%, compared to the control sample, during 5 days of storage.

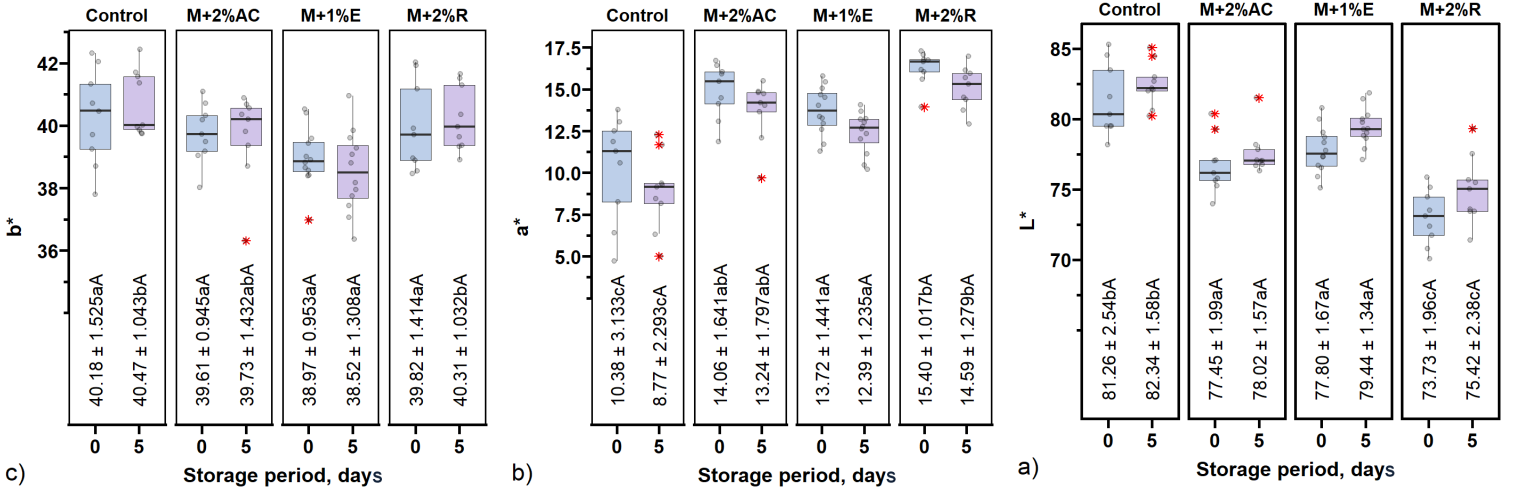

Fig. 3.4. Effect of rowanberry pomace ingredients on colour characteristics (*L**, *a**, *b**) on the day of preparation and after 5-day storage. Actual values are presented with grey dots; outliers are denoted with a red asterisk; mean values are presented as the least-square means ± standard deviations; different lowercase letters indicate the statistical difference ($p<0.05$) between the variants within the same day; different capital letters indicate the statistical difference ($p<0.05$) between the days within the same variant. Control – without plant-based ingredients; M+2%AC – meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace; M+1%E – meatballs with 1% of EtOH/water extract of AC; M+2%R – meatballs with 2% of extraction residue

Delta E (E – *Empfindung*, sensation), also written as ΔE or E*, is a significant standard measurement representing the total colour difference taking into consideration changes in the values of L^* as lightness, a^* as redness, and b^* as yellowness. Based on ΔE, it is possible to predict whether the changes in the meat colour are noticed by the consumer (CIE[211]).

Added plant ingredients had a significant effect on the total colour difference (ΔE_{Lab}) between the control and the test samples during the storage period. According to the results of the first experiment with hemp ingredients and sweet grass extract (**Fig. 3.5.**), even an unexperienced observer can notice the difference in colour ($\Delta E_{Lab} > 2$), especially regarding the samples with RHSG and SG ($\Delta E_{Lab} > 5$), where two different colours are noticed. It may be due to the presence of the dark green colour of sweet grass. It also indicates that consumers will perceive colour changes. Lighter green ingredients, DH and RH had a clear effect on the colour difference ($\Delta E_{Lab} > 2$) compared with the control sample. In conclusion, these results indicate that consumers will perceive colour changes of patties during chilled storage. As all the ingredients had an effect on ΔE_{Lab}, there is a need to find a method to decrease the colour-changing effect of the ingredients, especially in terms of developing new ready-to-eat products.

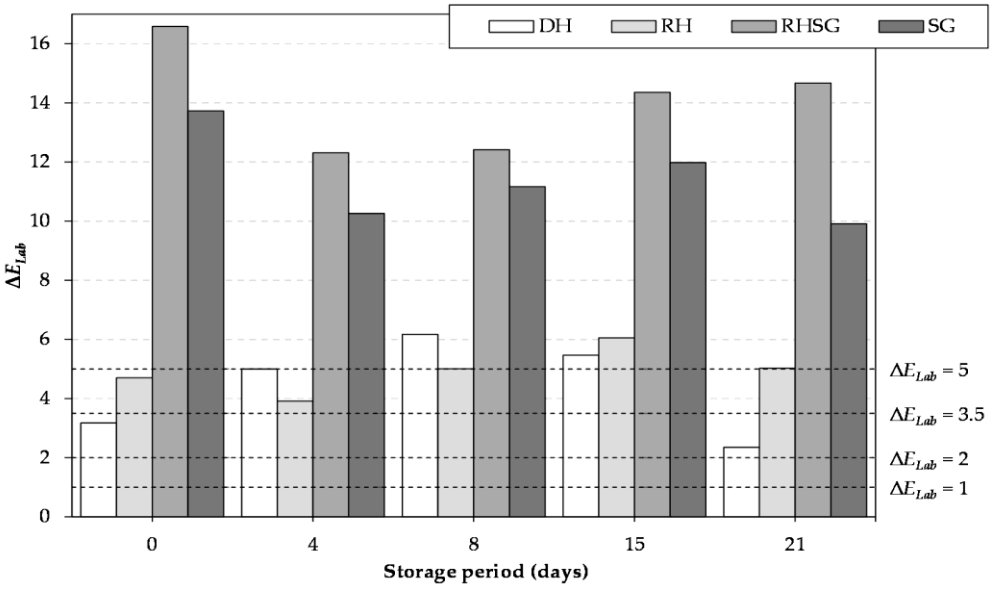

Fig. 3.5. Total colour difference (ΔE_{Lab}) between the control and test samples during the storage period: $0 < \Delta E_{Lab} < 1$ – the observer does not notice a difference; $1 < \Delta E_{Lab} < 2$ – only an experienced observer may notice the difference; $2 < \Delta E_{Lab} < 3.5$ – an unexperienced observer also notices the difference; $3.5 < \Delta E_{Lab} < 5$ – a clear difference in colour is noticed; and $5 < \Delta E_{Lab}$ – an observer notices two different colours (RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract and 1.5% dried pressed hempseed cake; SG – 0.5% sweet grass extract)

The addition of different BC seed ingredients affected the ΔE_{Lab} between the control sample and the samples with ingredients (**Fig. 3.6.**). It is obvious that there is a difference in colour ($\Delta E_{Lab} > 3.5$), especially in meatballs with BC-AE and BC-ASC. On day 3, some decrease in ΔE_{Lab} can be seen in samples BC-RS, but still a clear difference is noticed nevertheless. After 6 days of storage, the total colour difference was highest in BC-AE5. Again, consumers will perceive the colour changes during chilled storage, and the difference may be explained by the intensive colour of the added ingredients, and also due to the high content of anthocyanin pigments in BC.

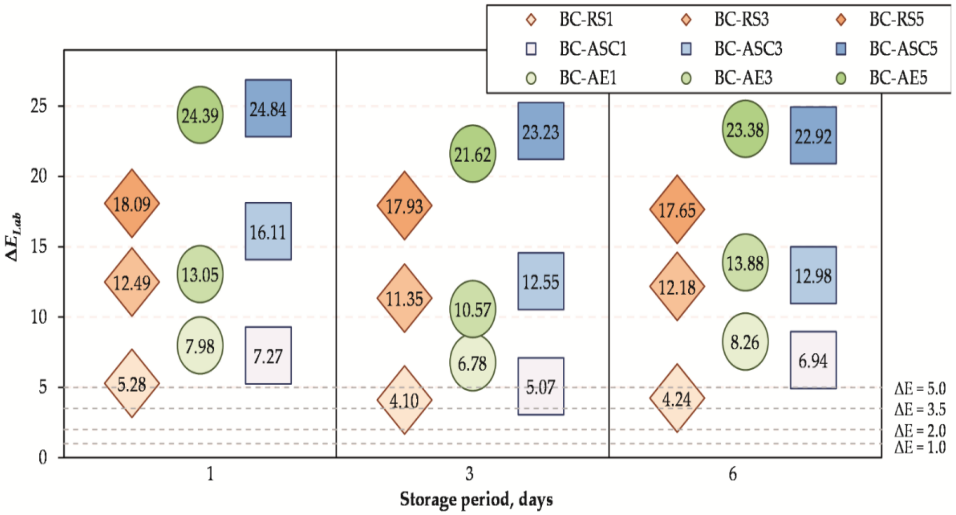

Fig. 3.6. Total colour difference (ΔE_{Lab}) between the control and samples with BC seed ingredients during the storage. $0 < \Delta E_{Lab} < 1$ – the observer does not notice a difference; $1 < \Delta E_{Lab} < 2$ – only an experienced observer may notice the difference; $2 < \Delta E_{Lab} < 3.5$ – an unexperienced observer also notices the difference; $3.5 < \Delta E_{Lab} < 5$ – a clear difference in colour is noticed; $5 < \Delta E_{Lab}$ – an observer notices two different colours. BC-RS – blackcurrant seeds before CO_2 extraction at concentrations 1, 3 and 5%; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction at concentrations 1, 3 and 5%; and BC-AE – blackcurrant seeds after EtOH/water extraction at concentrations 1, 3 and 5%

Notable perceived changes in the total colour difference ΔE_{Lab} were also detected in meatballs with rowanberry pomace ingredients (**Fig. 3.7.**). From the obtained results, it is clear that an observer notices two different colours in all samples during chilled storage, especially in meatballs with extraction residue (R), whose lightness (L^*) values were also lowest compared to other samples. It may again be explained by the colour of the added rowanberry ingredients containing anthocyanins and carotenoids producing the bright yellow, red, and orange colours.

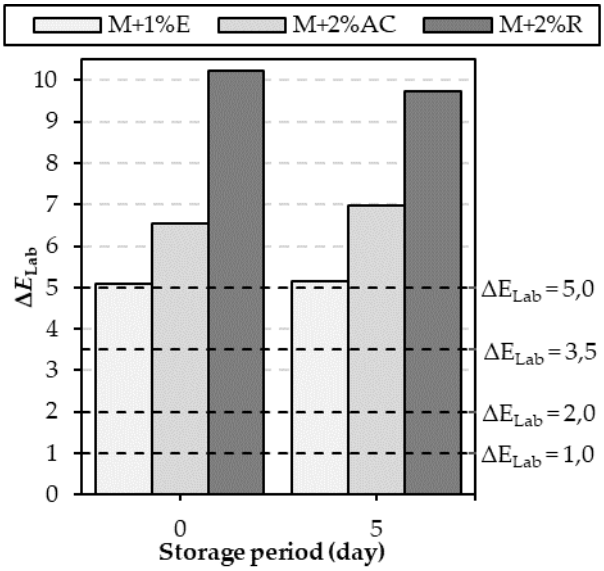

Fig. 3.7. Total colour difference (ΔE_{Lab}) between the control and samples with rowanberry pomace ingredients during the storage. $0 < \Delta E_{Lab} < 1$ – the observer does not notice a difference; $1 < \Delta E_{Lab} < 2$ – only an experienced observer may notice the difference; $2 < \Delta E_{Lab} < 3.5$ – an unexperienced observer also notices the difference; $3.5 < \Delta E_{Lab} < 5$ – a clear difference in colour is noticed; $5 < \Delta E_{Lab}$ – an observer notices two different colours. M+1%E – meatballs with 1% of EtOH/water extract of AC; M+2%AC – meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace; M+2%R – meatballs with 2% of extraction residue

3.2.3. pH changes during storage

pH is a crucial quality parameter of meat and meat products associated with other quality parameters, and it exerts influence on safety, shelf-life, sensory properties, colour intensity and water holding capacity. The decrease in pH can lead to an unacceptable taste, whereas, at the same time, it has an inhibitory effect against microbial spoilage (Barcenilla *et al.*[212]). Most bacteria grow optimally at approximately pH 7, and a high final pH in the final meat product has a potential for spoilage. Many studies have found that the use of plant-based or fruit-based ingredients in meat products causes a decrease in pH.

Table 3.12. shows the pH of grilled pork patties treated with hempseed ingredients and sweet grass extract during the storage period, ranging within 6.1–6.3. Sweet grass extract had no remarkable effect on this characteristic, while, evidently, hemp ingredients significantly increased the pH of pork patties after grilling and during the entire storage period. Kotecka-Majchrzak *et al.*[7] detected the increase in pH-values of meatballs with hemp cake after cooking. The higher values of pH with hempseed ingredients may be attributed to the addition of some small amount of buffer-type compounds which are present in hemp. The system is highly complex, and various processes may occur; therefore, it may be hypothesized that, during the storage of meatballs, proteins found in hempseed can be broken down due

to enzymatic processes, and the resultant amino acid may have alkaline characteristics, which possibly can cause the increase in pH. As hempseeds contain higher amounts of various minerals, such as magnesium and potassium, which are not solid bases, there might be some variations in the pH values of meatballs during the storage. Alkaline compounds found in hempseeds may become cleared in the measurement of pH, and may potentially cause the increase of pH in meatballs. The same effect was perceived and noted with the addition of amaranth and pumpkin seeds to chicken burgers (Longato *et al*.[26]). A small but significant ($p<0.05$) increase was observed in patties with RH after 15 and 21 days of storage, which may be hypothesized by the formation of acidic oxidation products of residual hemp oil.

It is relatively unlikely that the fluctuations in pH values have any noticeable effects on the other characteristics of the quality of meat products.

Table 3.12. Effects of ingredients and storage period (days) on the pH-value of grilled patties. Values are the least square means ± standard deviation.

Sample	Storage period (days)				
	0	4	8	15	21
Control	6.08 ± 0.15^{Aa}	6.09 ± 0.10^{Aa}	6.13 ± 0.08^{ABa}	6.11 ± 0.07^{Aa}	6.15 ± 0.09^{ABa}
DH	6.18 ± 0.09^{BCa}	6.22 ± 0.16^{Ba}	6.18 ± 0.10^{Ba}	6.24 ± 0.11^{BCa}	6.25 ± 0.12^{Ca}
RH	6.19 ± 0.08^{Cab}	6.21 ± 0.07^{Babc}	6.18 ± 0.13^{ABa}	6.29 ± 0.08^{Cc}	6.27 ± 0.13^{Cbc}
RHSG	6.18 ± 0.06^{BCa}	6.16 ± 0.13^{ABa}	6.17 ± 0.10^{ABa}	6.20 ± 0.08^{Ba}	6.20 ± 0.11^{BCa}
SG	6.11 ± 0.07^{ABa}	6.08 ± 0.08^{Aa}	6.10 ± 0.08^{Aa}	6.05 ± 0.09^{Aa}	6.08 ± 0.09^{Aa}

The least square means followed by the different capital letters in the columns and lower-case letters in the rows differ significantly by Tukey's multiple comparison's post hoc test ($p<0.05$). Control – without plant-based ingredients; RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract; 1.5% dried pressed hempseed cake; SG – 0.5% sweet grass extract

The effect of BC seed ingredients on the pH values of pork meatballs during 6 days of storage is described in **Fig. 3.8.** The added BC seed ingredients affected the pH values of meatballs. It is known that dietary fibre has an impact on the technological properties of meat products, while also changing the pH-value of systems (Sun *et al*.[197]). Also, Hautrive *et al*.[213] and Sánchez-Zapata *et al*.[214] noted the highest pH values with the added fibre-rich ingredients, likely due to the chemical characteristics of each fibre tested. The lowest pH after 3 and 6 days of storage was in the control sample (it was below 6.0), which tends to cause oxidation and a reduced water-holding capacity (Barrón-Ayala *et al*.[215]). In other samples, the pH values with BC seed ingredients remained the same. The decrease in pH may be due to the hydrolysis of lipids and the formation of free fatty acids. Many authors experimented with fruit and berries extract in meat products. Tamkutė *at al*.[171] found similar results in pH values, which slightly increased and afterwards remained the same, when adding chokeberry pomace extracts into pork burgers. The same was observed with the control sample whose pH decreased. Kumar and Kumar[216] treated chicken meat batter with encapsulated *Murraya koenigii* berries extract and

observed differences between the control and the experimental group. The values measured in the current study are also in line with Longato *et al.*[26], who measured pH between 6.1 and 6.6 in chicken burgers, or Heck *et al.*[217], who determined pH at a level of 6.2–6.4 in beef burgers.

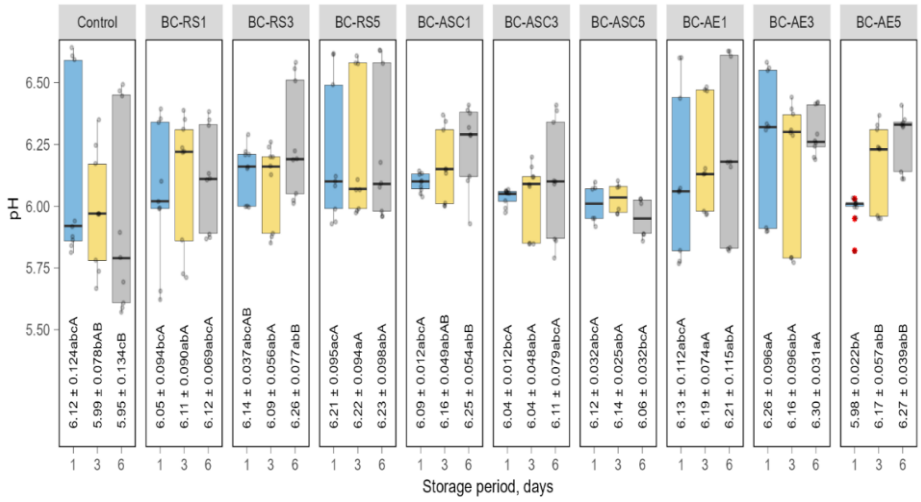

Fig. 3.8. Effect of BC seed ingredients and storage period (days) on the pH-value of cooked pork meatballs. Values are least square means ± standard error. The actual values are presented with grey dots, the outliers are marked with a red asterisk. Control – without plant-based ingredients; BC-RS – blackcurrant seeds before CO_2 extraction at concentrations 1, 3 and 5%; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction at concentrations 1, 3 and 5%; BC-AE – blackcurrant seeds after EtOH/water extraction at concentrations 1, 3 and 5%

The effect of rowanberry pomace ingredients on the pH-value of cooked pork meatballs is shown in **Fig. 3.9.** During the 5 days of storage, the pH value was significantly lower in the meatballs with 1%-E compared to the control sample, which may be explained by the presence of organic acids, such as chlorogenic acid, as also pointed out by Tamkutė *et al.*[171], when detecting lower pH-values in cooked ham with cranberry pomace. The pH values of meatballs with fibre-rich ingredients AC and R were the same and remained essentially the same during the 5 days of storage at 4 °C, which may be due to some content of chlorogenic acid, while the pH of the control sample increased, which refers to microbiological deterioration. Also, in the current research, during the OPLS-DA analysis, the main phenolic compound identified in meatballs with rowanberry ingredients was chlorogenic acid. On the other hand, Tamkutė *et al.*[171] found that the pH of cooked ham samples with chokeberry extract was constant during 36 days of storage at 4 °C, while the pH of the control sample increased. It was observed by the same author[218] that pork burgers with 2% ethanol extract had a lower pH compared to the control sample during storage at 4±1 °C, which can be explained by the low pH of the extract (2.5).

According to Halagarda and Wójciak[219], perishable meat products (a_w > 0.95 and pH > 5.2) must be stored at or < 5 °C.

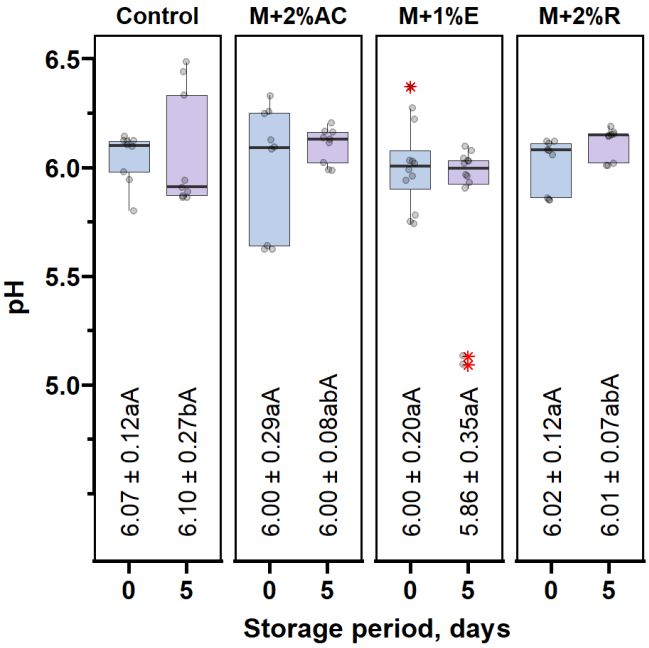

Fig. 3.9. Effect of rowanberry pomace ingredients on the pH-value of cooked pork meatballs on the day of preparation and after five days of storage. The actual values are presented with grey dots; outliers are denoted with a red asterisk; the mean values are presented as the least-square means ± standard deviations; different lowercase letters indicate statistical difference ($p<0.05$) between variants within the same day; different capital letters indicate statistical difference ($p<0.05$) between days within the same variant). Control – without plant-based ingredients; M+2%AC – meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace; M+1%E – meatballs with 1% of EtOH/water extract of AC; M+2%R – meatballs with 2% of extraction residue

3.2.4. Water activity (a_w) changes during the storage

Water activity (a_w) indicates the ration of the vapour pressure of water to a vapour pressure of pure water, available for microorganisms to grow. Together with pH, they are important factors of the microbial growth and oxidation of meat products, and also in terms of product stability during the storage. The a_w value of meat products is usually high for various microbiological and (bio)chemical processes.

The a_w values ranged during the 21 days of storage period within 0.950–0.963 (**Tbl. 3.13.**). Again, as well as with changes in the pH-level, some slight differences in the samples with hemp press-cake and sweet grass extract were detected, although ANOVA indicated significantly higher values for almost all stored samples with

added ingredients compared with the control sample. Kotecka-Majcharzak *et al.*[7] observed a similar pattern of a_w-values in meatballs (stored in refrigerated condition) incorporated with hemp cake. In their study, the water activity of meatballs was in the range between 0.9770 and 0.9803.

Table 3.13. Effects of ingredients and storage period (days) on the a_w-value of grilled patties. Values are the least square means ± standard deviation.

Sample	Storage Period (days)				
	0	4	8	15	21
Control	0.953 ± 0.022^{Aa}	0.950 ± 0.022^{Aa}	0.950 ± 0.021^{Aa}	0.951 ± 0.021^{Aa}	0.952 ± 0.016^{Aa}
DH	0.957 ± 0.022^{Aa}	0.953 ± 0.019^{ABa}	0.956 ± 0.018^{Ba}	0.957 ± 0.018^{BCa}	0.955 ± 0.018^{ABa}
RH	0.957 ± 0.017^{Aa}	0.957 ± 0.018^{Ba}	0.957 ± 0.019^{Ba}	0.955 ± 0.021^{ABa}	0.956 ± 0.017^{ABa}
RHSG	0.963 ± 0.022^{Ba}	0.963 ± 0.022^{Ca}	0.964 ± 0.020^{Ca}	0.961 ± 0.022^{Ca}	0.963 ± 0.016^{Ca}
SG	0.958 ± 0.020^{Aa}	0.957 ± 0.022^{Ba}	0.959 ± 0.021^{Ba}	0.957 ± 0.021^{BCa}	0.961 ± 0.017^{BCa}

The least square means followed by the different capital letters in the columns and lower-case letters in the rows differ significantly by Tukey's multiple comparison's post hoc test ($p<0.05$). Control – without plant-based ingredients; RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract and 1.5% dried pressed hempseed cake; SG – 0.5% sweet grass extract

The a_w-values of meatballs with BC seed ingredients (**Fig. 3.10.**) ranged within 0.978–0.984 during the 6 days of storage and were quite stable within the samples and storage days. Similar results were obtained by Tamkutė *et al.*[171], where the water activity in cooked ham with chokeberry extract was between 0.978–0.983, and, for raw burgers, it measured 0.988−0.997 within the storage period. Jaberi *et al.*[220] found out that the addition of barberry (*Berberis vulgaris* L.) extract had no effect on the a_w-value of frankfurters. Some decrease in a_w-values during the storage, as determined in the current study, was observed in samples BC-RS1, BC-RS3, and BC-RS5, but such a decrease does not have any remarkable effect on different processes in meatballs. Any significant effect on a_w was also not detected by Tamkutė *et al.*[218] in meat products with cranberry pomace ethanol extracts, either.

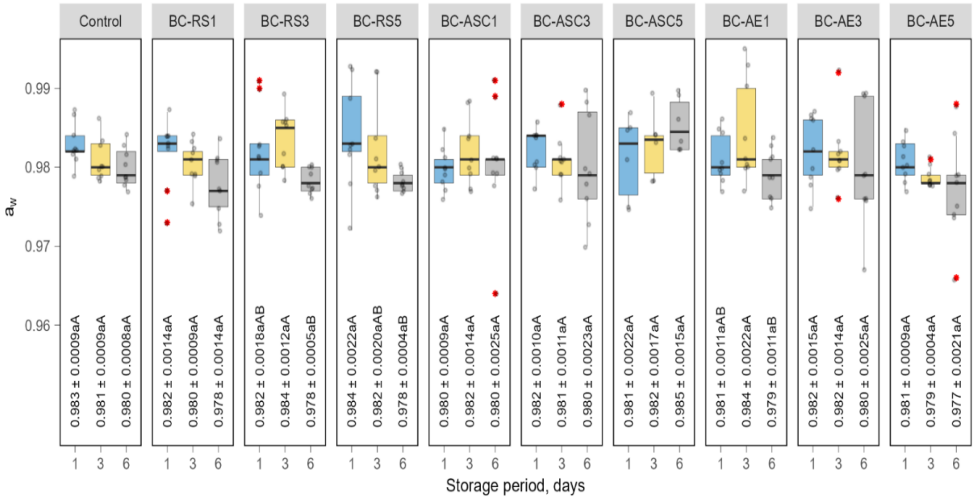

Fig. 3.10. Effect of BC seed ingredients and storage period (days) on the a_w-value of cooked pork meatballs. Values are the least square means ± standard error. The actual values are presented with grey dots, the outliers are given with a red asterisk. Control – without plant-based ingredients; BC-RS – blackcurrant seeds before CO_2 extraction at concentrations 1, 3 and 5%; BC-ASC – blackcurrant seeds after supercritical fluid CO_2 extraction at concentrations 1, 3 and 5%; BC-AE – blackcurrant seeds after EtOH/water extraction at concentrations 1, 3 and 5%

With rowanberry pomace ingredients, the measured a_w-values of meatballs ranged within 0.974–0.987 (**Fig. 3.11.**). While the pH of meatballs with fibre-rich ingredients AC and R remained the same during the 5 days of storage, then, in a_w-values, the highest reduction (1.3%) was detected in meatballs with the addition of extraction residue (R). Yet, such a decrease does not remarkably affect the shelf-life of meatballs. The addition of 2%-AC and 1%-E did not have any significant effect on the changes in the a_w-values, as the first variant had the same values as those for the control sample during the storage. Similarly, Tamkutė *et al.*[171] noticed only a marginal effect on a_w in the case of cranberry pomace ethanol extract addition.

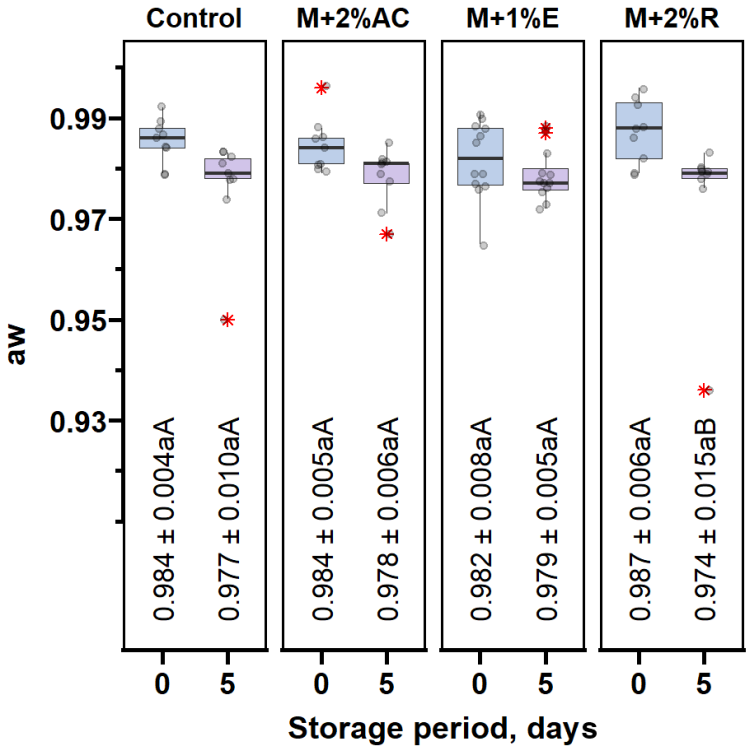

Fig. 3.11. Effect of rowanberry pomace ingredients on the a_w-value of cooked pork meatballs on the day of preparation and after five days of storage. The actual values are presented with grey dots; the outliers are denoted with a red asterisk; the mean values are presented as the least-square means ± standard deviations; different lowercase letters indicate statistical difference ($p<0.05$) between variants within the same day; different capital letters indicate statistical difference ($p<0.05$) between days within the same variant). Control – without plant-based ingredients; M+2%AC – meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace; M+1%E – meatballs with 1% of EtOH/water extract of AC; M+2%R – meatballs with 2% of extraction residue

3.2.5. Effect on the oxidation of pork meat products

Oxidation is one of the most important harmful processes in the processing and storage of food, especially in meat. Oxidation, in a broad sense, is a chemical process, during which, electrons are taken from one chemical particle (for example, a molecule), and they join another particle, whereby the first particle is oxidized and the second particle is reduced. Lack of antioxidants, a high content of PUFAs content, heat and light during processing, etc. – all of these promote oxidation in meat. Lipid oxidation causes rancidity, a shorter shelf-life, changes in flavour, colour, nutritional value, etc. To prevent, delay, or slow down the oxidation processes, bioactive compounds, known as antioxidants, are most widely used in meat products.

The method of TBARS (thiobarbituric acid reactive substances), used as a rapid measurement for lipid oxidation, is based on visible light absorption. The initial values in the current research were conventionally equalized to 0, and later the changes during storage were monitored. The higher is the TBARS assay value, the higher is the level of lipid oxidation. It is explicitly clear that, due to sweet grass being a strong antioxidant (**Tbl.e 3.2.**), its extract fully stabilized the formation of TBARS during the whole period of storage (**Fig. 3.12.**). It can be explained by the content of 5,8-dihydroxycoumarin to have great antioxidant activity, which has also shown a good effect in retarding rapeseed oil oxidation (Slapšytė *et al*.[81]). The TBARS assay values increased in control samples after 21 days from 0.420 to 0.540 mg/MDA/kg, while that in pork patties with DH and RH increased from 0.197 to 0.297, and from 0.181 to 0.364 mg/MDA/kg, respectively.

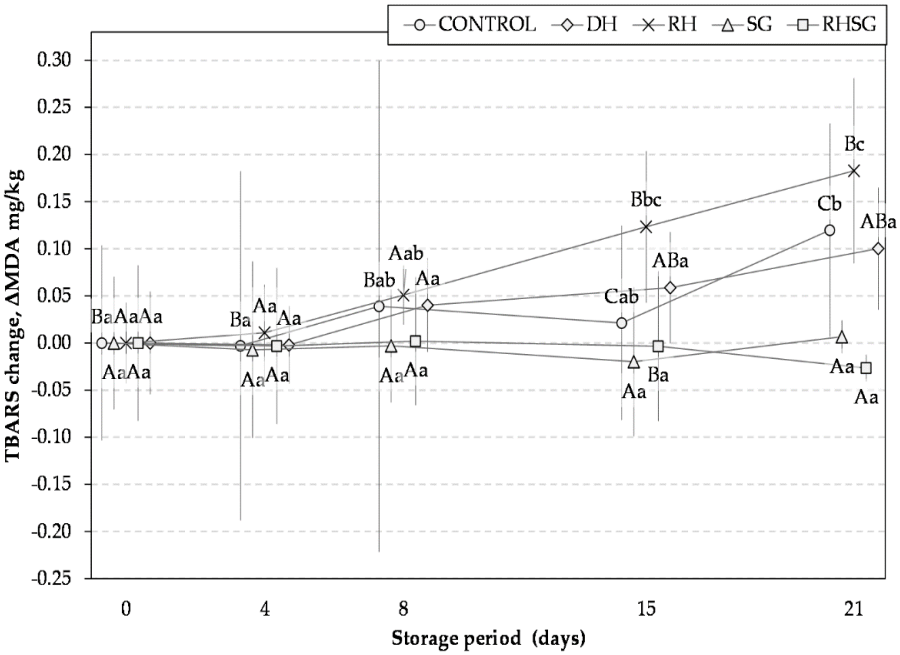

Fig. 3.12. Changes in TBARS assay values of grilled pork burger patties stored in modified atmosphere during the storage period (ΔMDA mg/kg). Different capital letters express a significant difference between the variants within the same storage day by Tukey's test ($p<0.05$). Different lower-case letters express a significant difference between storage days within the same variant by Tukey's test ($p<0.05$). Control – without plant-based ingredients; RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract and 1.5% dried pressed hempseed cake; and SG – 0.5% sweet grass extract

The highest TBARS assay values were detected in the samples with raw hempseed cake (RH) after 15 and 21 days of storage, which supports the hypothesis that unsaturated oil residues in the raw hempseed press-cake may foster the formation of oxidation products. A high increase in oxidation products after 15 days of storage was also noticed by Zajac *et al.*[22] in pork loaves with de-hulled hempseed

and in the control sample. In other samples, the TBARS was stable as the hemp protein and flour are by-products of fat extraction. When sweet grass (SG) was applied together with RH, the formation of malondialdehyde (MDA) as a highly toxic secondary oxidation product, was fully inhibited. It may be again explained by the addition of SG containing a strong antioxidant (specifically, 5,8-DHC), which stabilized the oxidation of highly unsaturated oils present in RH. No significant changes were detected in the TBARS assay values during the whole period of storage in the sample SG – as it stayed in the range between 0.160–0.186 mg/MDA/kg. When using defatted hempseed protein press-cake (DH), the formation of TBARS was largely similar to that in the control sample.

Part of our research (unpublished data) was to test the effect of BC skin ingredients on the oxidation of meatballs. The addition of BC skin ingredients had a positive effect on the reduction of lipid oxidation in meatballs. As berries contain several phenolic compounds, the antioxidant effect may be related to the phenolic content of berry pomace extracts. It is known that seeds accumulate a larger amount of protein and oil, whereas most phenolic compounds are accumulated in the skin of fruits (Kerner *et al*.[176]; Mäkilä *et al*.[221]). **Fig. 3.13** illustrates the effect of BC skin ingredients and the storage time on the MDA concentration in meatballs during 14 days of refrigerated storage. It is evident that, in the control sample, oxidation increased significantly during the storage period compared to the samples with BC skin ingredients. Those results are also in line with the results of the amount of hexanal (a volatile aldehyde) (**Fig. 3.14.**) as an indicator of oxidative stability. It should be noted that, on the 7th day of storage, the TBARS assay values increase in all samples with BC skin ingredients, but, by the end of the final day of storage, it decreases in all samples. It may be hypothesized that antioxidants, which are present in BC skins, may be exhausted during the storage, while oxidation processes become more intensive. The lowest TBARS assay values were observed in meatballs with AA, followed by RS. As the system is very complex, therefore, different effects may be expected from various ingredients. These effects may also involve some antagonistic character. The ingredients after CO_2 extraction still contain some PUFA-rich oil, which may be oxidized to various products. For HOE, it may be assumed that EtOH/water extraction removed hydrogen donating polyphenolic antioxidants, and it can be hypothesized that the presence of polyphebnolic antioxidants is not able to prevent the formation of some oxidation products. Fourati *et al.*[222] studied pomegranate (*Punica granatum*) peel extracts for the preservation of minced beef and identified that 1% of aqueous peel extract decreased the lipid oxidation during the storage for 21 days at 4 °C.

These results clearly showed that the use of BC skin ingredients as natural antioxidant sources could be effective in protecting meatballs from lipid oxidation, compared to the control sample, during storage. The inhibitory effect on lipid oxidation is attributed to its phenolic compounds, and therefore radical chain reactions get blocked in the oxidation process.

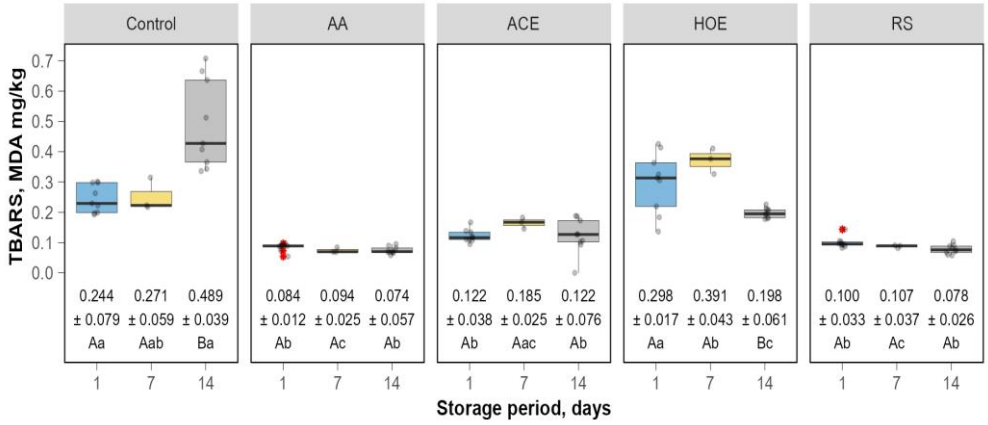

Fig. 3.13. Changes in the TBARS assay values of cooked pork meatballs with the addition of 2% of blackcurrant skin ingredients stored in modified atmosphere during the storage period. Values are the least square means ± standard error. The actual values are presented with grey dots; the outliers are given with a red asterisk. Control – without plant-based ingredients; RS – with 2% of raw blackcurrant pomace skins; ACE – with 2% of blackcurrant pomace skins after CO_2 extraction; HOE – with 2% of combined extract of defatted residues consecutively isolated with EtOH and H_2O; AA – with 2% of blackcurrant pomace skins after all extractions

As the discolouration of meat products is related with various substances formed during the oxidation processes, added ingredients inhibited the oxidative reactions, and therefore they may also protect from discolouration during the storage.

In conclusion, according to Wood *et al.*[223], the values up to 2.00 mg of MDA/kg product are not perceived by the consumers, and therefore none of the tested samples reached this value during the storage period.

Volatile lipid oxidation products, also known as secondary oxidation products, are responsible for flavour and taste deterioration. Hexanal, as a secondary product of lipid oxidation, was identified as the major volatile aldehyde generated from lipid peroxidation; headspace techniques are used to measure volatile components. **Fig. 3.14.** shows part of the unpublished data for the variation in the absolute amount of hexanal in cooked meatballs with BC skin ingredients, analyzed by head space solid phase microextraction gas chromatography with time-of-flight mass spectrometry (HS-SPME-GS-TOF). The hexanal content was expressed in arbitrary units (au), which means the total ion current of the relevant peaks as the meatball samples were sufficiently solid state. Therefore, to inject the appropriate internal standard and distribute it evenly throughout the all volume, was a prohibitively complicated task. The obtained information is sufficient because the hexanal peak area is proportional to its concentration in the product. A high increase in the hexanal content was detected in the control sample (without BC skin ingredients). After 14 days of storage, all the samples with BC skin ingredients featured an increased headspace

hexanal level, but it was still lower than in the control sample. The meatballs with rich in antioxidant polyphenols AC and HOE demonstrated the lowest hexanal level.

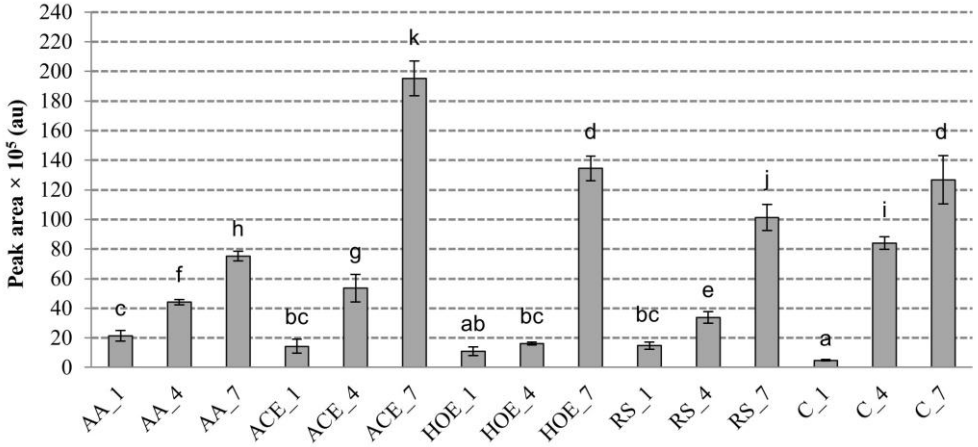

Fig. 3.14. Variation in the absolute amount (in arbitrary units, au × 10^5) of hexanal in the headspace of cooked meatballs with BC skin ingredients during the storage period at +4 °C. Control – without plant-based ingredients; RS – with 2% of raw blackcurrant pomace skins; AC – with 2% of blackcurrant pomace skins after CO_2 extraction; HOE – with 2% of combined extract of defatted residues consecutively isolated with EtOH and H_2O; AA – with 2% of blackcurrant pomace skins after all extractions

3.2.6. Sensory evaluation

Nowadays, consumers prefer healthier products with good sensory characteristics, which is why it is important that plant-based ingredients would not have any negative effect on the sensory quality of meat products. Therefore, the determination of acceptable doses and their effects on several organoleptic characteristics remains an important issue. Zajac *et al*.[22] stated that if the green colour detected in meat products is from known sources, it does not lower the consumers' expectations.

Therefore, assessing the effects of the selected ingredients on the sensory quality parameters of raw and grilled pork patties was among the most important tasks of the study with hemp press-cake ingredients and sweet grass extract (**Fig. 3.15.** and **Fig. 3.17.**). The sensory evaluation of pork patties was performed by assessing their appearance, colour, odour, taste and juiciness on the 9-point hedonic scale. This approach has been quite widely used for comparison purposes, particularly in the case of using new ingredients (Tuorila and Monteleone[224]), and therefore it can provide important information for the further product development.

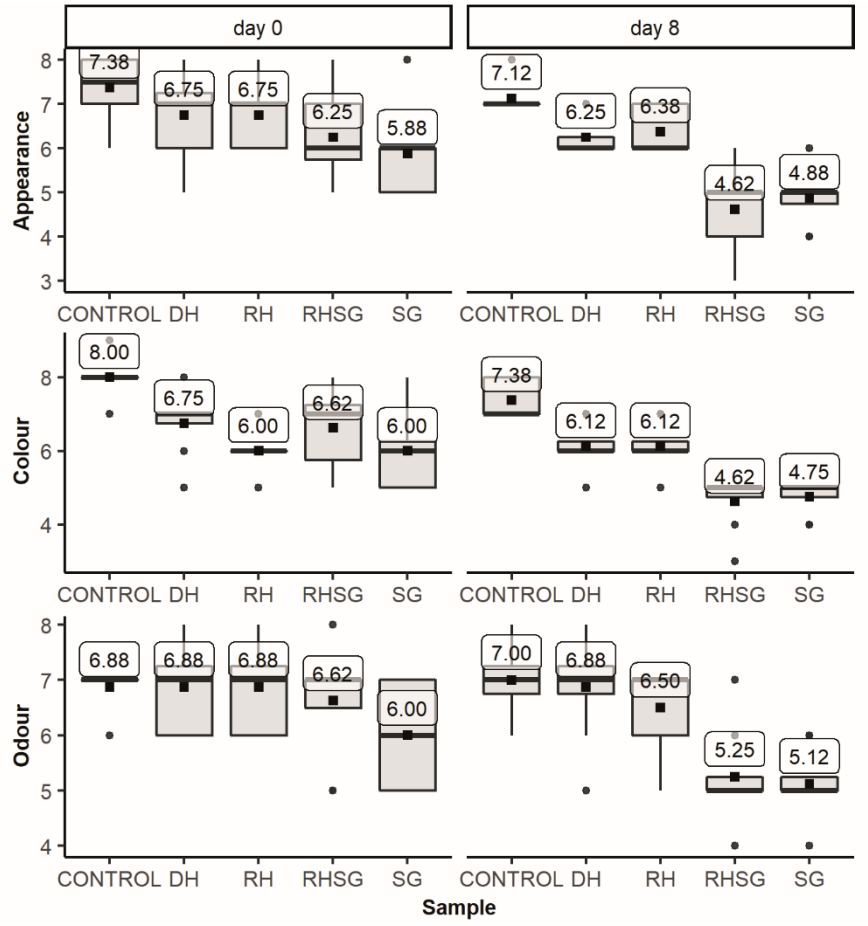

Fig. 3.15. Sensory characteristics of raw pork patties with various plant-based ingredients on storage days 0 and 8 on the 9-point hedonic scale (control – without plant-based ingredients; RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract and 1.5% dried pressed hempseed cake; SG – 0.5% sweet grass extract)

SG extract affected the most the sensory parameters of pork patties in the case of raw and grilled ones; therefore, samples with SG and RHSG received the lowest scores from the panellists for all the assessed parameters, except for juiciness being the lowest in the control sample. Grilled pork patties with the addition of sweet grass extract are shown in **Fig. 3.16.** The appearance and colour were assessed with the highest scores for the control sample, and the colour was also determined by the spectrophotometric method (**Tbl. 3.8.**), while the dark-green SG extract reduced the lightness by 16–20%. Based on the hedonic 9-point scale used, score 5 was set as 'satisfying', and all the samples assessed received the score for the odour above the

acceptability limit, although, in samples RHSG and SG, it decreased after 8 days of storage.

Fig. 3.16. Grilled pork patties with the addition of sweet grass extract

To conclude, the results for raw patties showed the highest scores for the control samples followed by DH, RH, and SG ingredients demonstrating that the lighter and the more familiar are the appearance and the colour of the product, the more acceptable the product is.

In the case of grilled patties (**Fig. 3.17.**), the variability of the evaluation scores was observed for some sensory characteristics. During the sensory evaluation, the assessors pointed out the unusual green colour, especially in patties with SG receiving also the lowest scores for odour, with an average of 5.3. For juiciness, samples with SG got higher ratings compared to samples without any additives. The ratings for patties with SG were quite uniform (62.5% of the assessors gave a score of 5). The lowest score for the appearance was assigned to patties with RHSG on days 0 and 8; the values were 6.12 and 4.88, respectively. For the patties with hempseed ingredients, the results were somewhat similar, as, for all the evaluated characteristics, the values ranged between 6 and 7. For those patties, a specific acceptable nutty odour was pointed out, which also had a positive effect on juiciness, especially in DH compared to the control sample. Although, the sensory assessment results in the experiment by Zajac *et al.*[22] pointed out that the juiciness and taste for the control sample was better (as it received higher scores) compared to the samples with the addition of 5% of hemp ingredients. It may be explained by the different heat treatment method and the concentration of the ingredients being used. It is also stated that, to some extent, the products with hemp ingredients are not acceptable for consumers. On the other hand, if the information about the healthiness of the product is provided, consumers may be willing to buy it. Consumer survey was also done by Zajac *et al.*[22] in their research, and the results concluded that, if the product is sensorically not acceptable and as consumers mostly pay attention to labels, there is a chance to bring such products into the market.

According to the current research, patties with DH and RH received relatively high scores for their appearance, colour and taste, which indicates that those protein-

rich hempseed press-cake ingredients are acceptable for consumers and could be further used in the development of meat products.

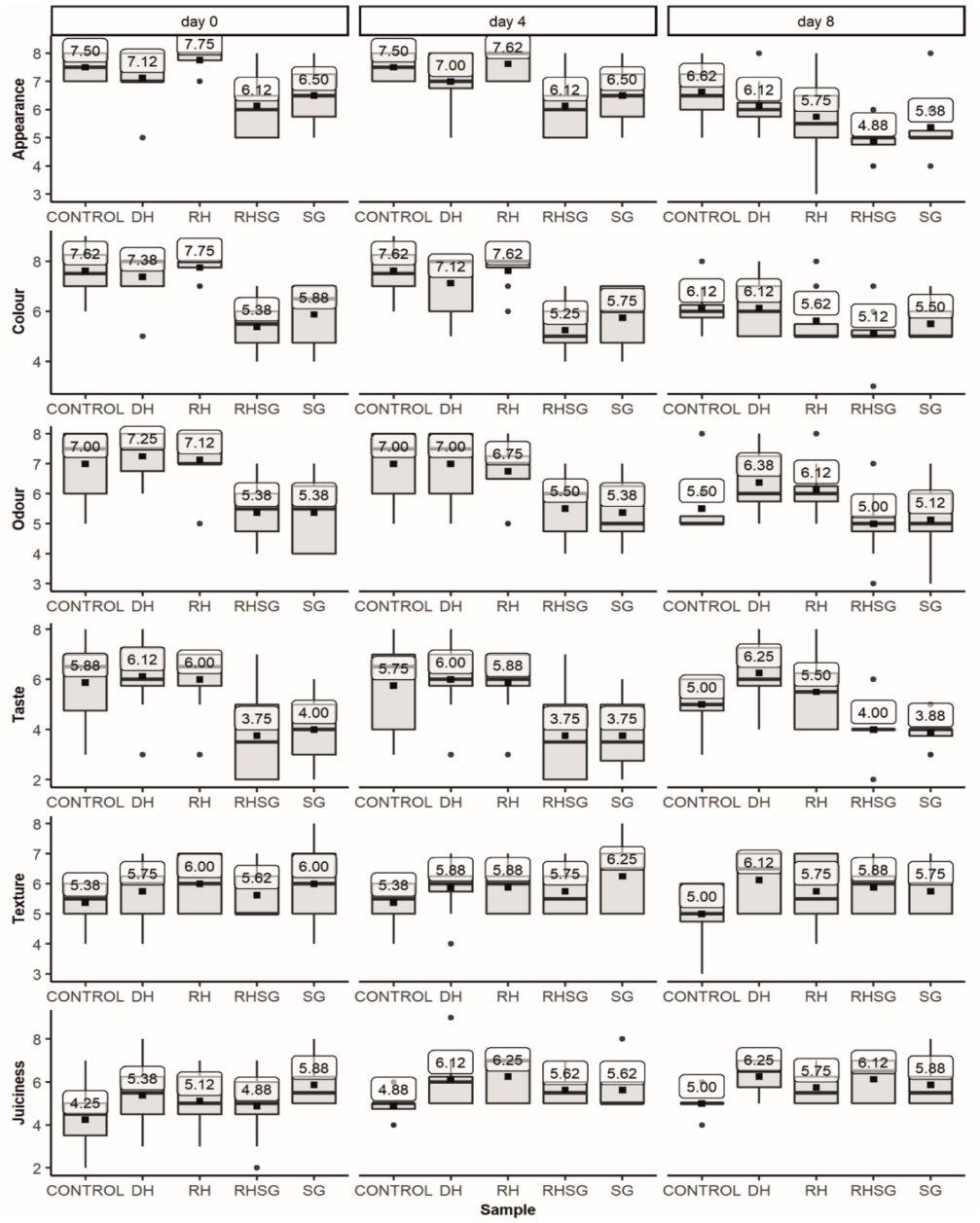

Fig. 3.17. Sensory characteristics of grilled pork patties with various plant-based ingredients on storage days 0, 4, and 8 on the 9-point hedonic scale (control – without plant-based ingredients; RH – with 2% dried mechanically pressed hempseed cake; DH – with 2% defatted by supercritical CO_2 extraction hempseed cake; RHSG – with 0.5% sweet grass extract and 1.5% dried pressed hempseed cake; SG – 0.5% sweet grass extract)

Applying plant-based ingredients into food products as preservatives or antioxidants may be limited due to the sensory characteristics. The major obstacle in terms of using rowanberry, which is generally widely grown, in food matrixes is its astringent taste. Therefore, also in this research, it was of importance to know the sensorically acceptable dose and the effect on several organoleptic characteristics. For the preliminary sensory evaluation, concentrations from 1 to 5% of rowanberry pomace ingredients in meatballs were tested (**Fig. 3.18.**), and the most acceptable dose for each group was selected. Preliminary sensory screening of the newly developed products by a smaller number of panellists is a commonly used practice to determine a formula which would be acceptable for the consumers (Bortolotto *et al.*[225]; Gonzalez *et al.*[226]; Michalczyk *et al.*[227]; Sam *et al.*[228]). The control sample without any ingredients was assessed as a reference. The sensory evaluation of pork meatballs was performed by assessing their appearance, colour, odour, taste, and juiciness.

The best score for the taste was given to 1%-R (7.5), which is also most acceptable in the case of odour. The samples with more than 1% of EtOH/water extract of AC were not acceptable for the assessors; therefore, higher concentrations were excluded from further experiments. As the samples AC and R with concentrations 1–3% received higher scores (more than 5 [which stands for 'satisfying']), 2%-AC and 2%-R as the average concentration was chosen for further experiments.

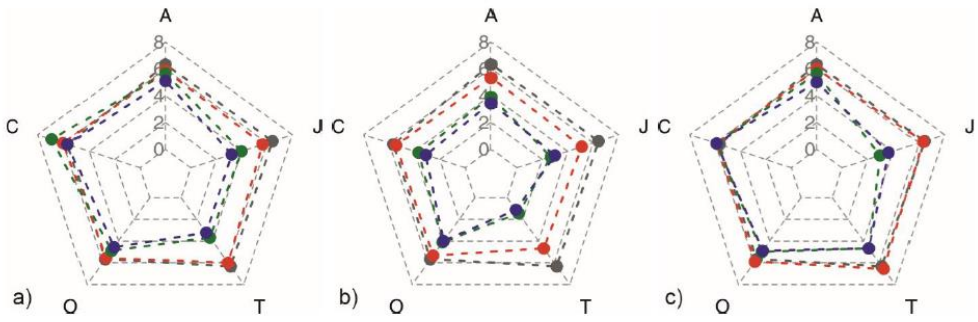

Fig. 3.18. Hedonic 9-point scale scores of panellists for the sensory acceptance of meatballs. A – appearance; C – colour; O – odour; T – taste; J – juiciness; a) **Control**, **M+1%AC**, **M+3%AC**, **M+5%AC**; **b) Control**, **M+1%E** , **M+3%E**, **M+5%E**; **c) Control**, **M+1%R**, **M+3%R**, **M+5%R.** Control – without ingredients; M+2%AC – meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace; M+1%E – meatballs with 1% of EtOH/water extract of AC; M+2%R – meatballs with 2% of extraction residue

3.2.7. Changes of the chemical profile of pork meat products

To evaluate the product quality and the effect of plant-based ingredients on the composition of small molecules, non-targeted metabolomics was used. This approach is *not* commonly used for assessing oxidation processes in meat. This analysis allowed to identify 749 metabolites in meatballs with BC seed ingredients.

In general, the enrichment analysis built considering the annotated meat metabolites showed a great abundance of amino acids and peptides (65 compounds), followed by flavonoids (43 compounds), fatty acids and conjugates (27 compounds), prenol lipids (22 compounds), and fatty esters (20 compounds). Also, a marked distribution of phenolic compounds, likely deriving from BC seeds, was revealed. The most represented class of polyphenols was that of flavonoids, with a great abundance of anthocyanins (such as glycosylated forms of cyanidin, petunidin, and delphinidin), flavones, flavonols, and other compounds. Other annotated classes were represented by phenolic acids (*e.g.*, gallic, chlorogenic, and sinapic acids), stilbenes, lignans, and tyrosol-derivatives. According to the scientific literature, BC seeds are a good source of bioactive phenolics (Basegmez *et al.*[142]) Among the annotated compounds, several primary and secondary oxidation markers were detected, *e.g.* 13-L-hydroperoxylinoleic acid, (12*S*,13*S*)-epoxylinolenic acid, 9,10-epoxyoctadecenoic acid, glutathione, glutathione disulfide, L-carnosine, and L-ascorbic acid.

Unsupervised hierarchical cluster analysis (**Fig. 3.19.**) clearly separated the control sample from the meatballs with the addition of various inclusion levels of RS, AE, and ASC ingredients.

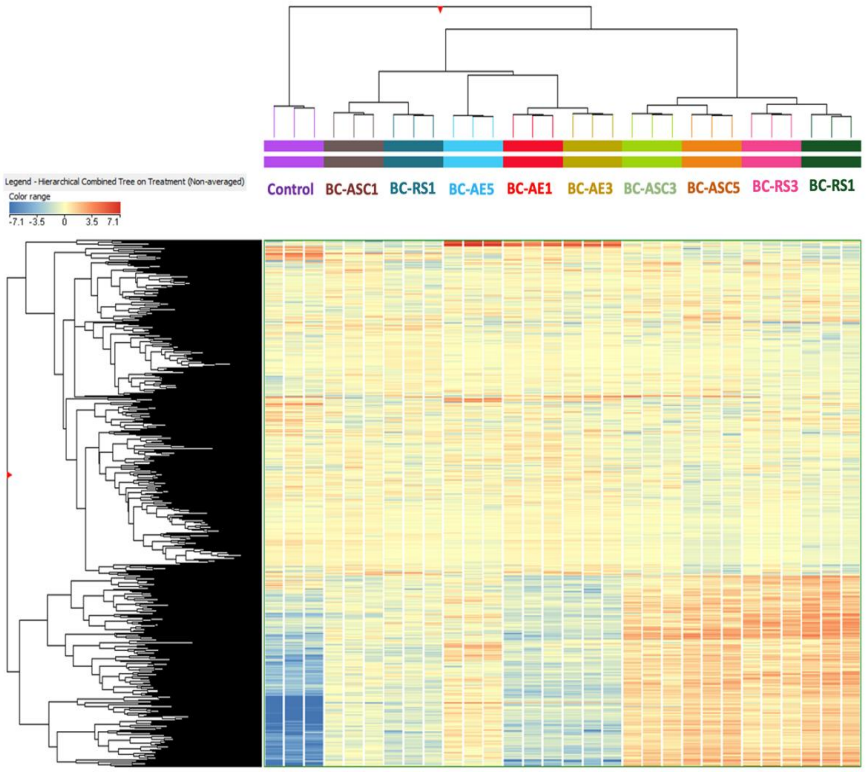

Fig. 3.19. Unsupervised hierarchical cluster analysis built considering the compounds annotated by untargeted metabolomics in various meatballs: different inclusion levels of BC-based ingredients

A similar chemical profile was found to have meatballs formulated with BC-AE1, BC-AE3, and BC-AE5. Taken together, unsupervised statistics declared that the most efficient treatments capable of significantly changing the chemical profile of meatballs were those based on the addition of high levels (3–5%) of BC seeds before and after supercritical fluid CO_2 extractions.

Multivariate statistical analyses were used to describe the impact of different BC-seed ingredients on the metabolite profile of the formulated meatballs. A different chemical profile was provided after each inclusion level of BC seed ingredients (**Fig. 3.20A, B**, and **C**) compared with the control sample. The most specific chemical profile could be obtained when using the maximum inclusion level (5%), while lower inclusion levels (1 and 3%) ultimately determined few chemical differences.

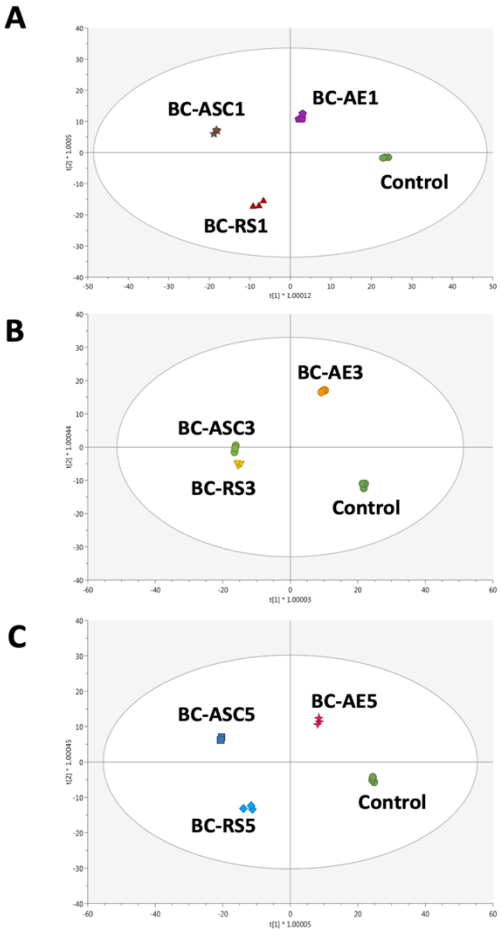

Fig. 3.20. OPLS-DA models considering different inclusion levels of BC-functional ingredients (*i.e.*, BC-RS, BC-ASC, and BC-AE) in pork meatballs. [A] = 1% inclusion; [B] = 3% inclusion; [C] = 5% inclusion

From the chemical point of view, a defatted BC seed ingredient (BC-ASC) was very similar to the initial material, raw seeds (BC-RS), but containing a small amount of residual lipids. Also, three discriminant anthocyanins, resulting from the inclusion of 1%-BC functional ingredients, were found, especially in samples BC-ASC (1%) compared with BC-RS and BC-AE pork meatballs. Lipid peroxidation marker (11R,12*S*,13*S*)-Epoxy-hydroxyoctadeca-*cis*-9-*cis*-15-dien-1-oic acid was found to decrease only in pork meatballs with 1% of BC-AE (LogFC = -0.46). Though, ingredient BC-AE is the residue after multiple extractions. Probably this role is exerted by some antioxidant compounds characterizing the extract. Still, these results must be considered as preliminary and indicative, and we must also bear in mind that the untargeted metabolomics approach was used, which provides only semi-quantitative results; thus it is better to focus on the total classes of compounds rather than on a single biomarker. The 1% inclusion level of the different BC ingredients determined a significant down-accumulation of α-linolenic acid (VIP score = 1.11), with an average LogFC value = -2.84.

Regarding the 3% inclusion level, the variation was lower, thus indicating a lower efficiency of these ingredients to counteract oxidative stress at this inclusion level. When looking at lipid oxidation marker chemicals, 3% BC-AE was the most successful component in lowering the amount of oxidation-related derivatives.

Finally, with the inclusion of 5% of BC seed ingredients, in total, 231 discriminant compounds were in common between the 3% and 5% inclusion levels, which indicates that an increased inclusion percentage of cooked pork meatballs may have a comparable effect on their metabolomic profile. Overall, lipid peroxidation markers showed similar trends compared to the prior inclusion levels. A significant and proportional rise in 13-L-hydroperoxylinoleic acid was determined, with BC-ASC supplemented sample showing the highest increase (LogFC = 4.97).

To conclude, both the percentage of inclusion and the extraction method used are crucial factors that need to be carefully evaluated in order to improve the overall quality of the cooked pork meat products.

For the next untargeted chemical profiling with rowanberry pomace ingredients, unsupervised hierarchical cluster analysis (HCA) was used to group samples according to intrinsic similarities in their chemical profile. A corresponding heat-map (based on the Fold-Change, FC, and variations of each annotated compound) is presented in **Fig. 3.21.**, consisting of 2 groups: the first cluster includes the control samples at the different time-points of storage (*i.e.*, 0, 4, and 14 days), while, in the second cluster, all the meatballs were prepared with the different pomace ingredients (*i.e.*, 2%-AC, 1%-E, and 2%-R). The heat-map highlights the potential effect of rowanberry pomace ingredients on the metabolomic profile of meatballs. A clear separation between the control samples and the meatballs with added ingredients was obtained. The impact of the storage time was evident, especially when considering 2%-AC and 1%-E added samples.

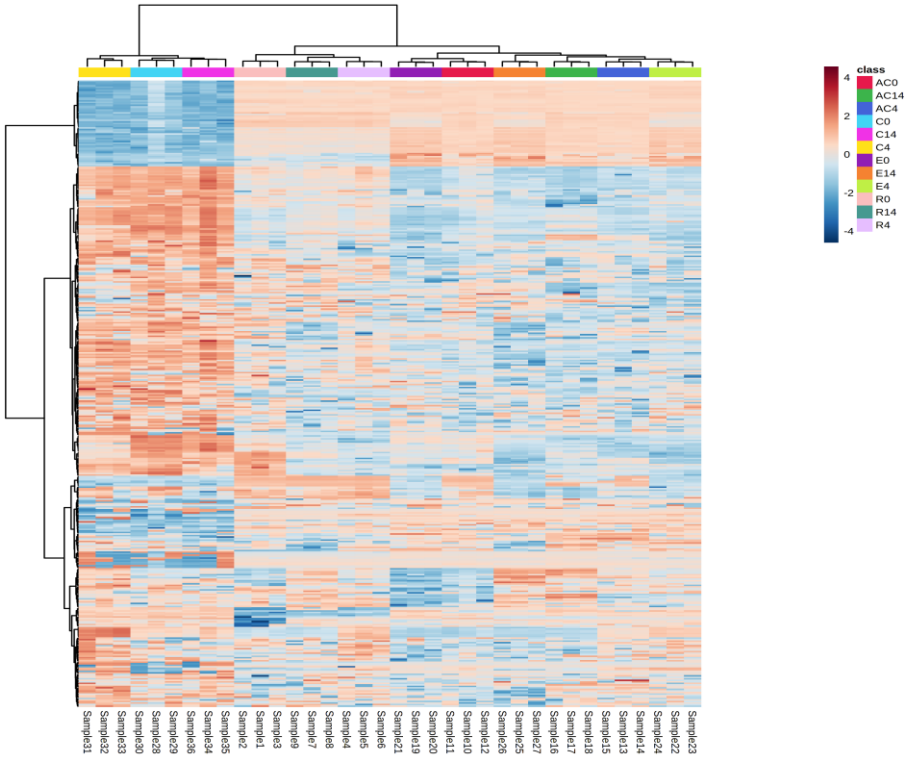

Fig. 3.21. Unsupervised hierarchical cluster analysis (HCA) considering the chemical profile of different meatballs prepared with rowanberry functional ingredients (AC, E, and R) vs. the control (C) at different storage time-points (*i.e.*, 0, 4, and 14 days)

Considering the last storage time point, that is, day 14, changes in the meat metabolites were evaluated in order to obtain more information about the potential impact of oxidation processes and the impact of rowanberry pomace ingredients. The period of 14 days of storage was previously studied in various articles, *e.g.* by Song *et al.*[229], or by Stobnicka *et al.*[230]. For that, an OPLS-DA model was built (**Fig. 3.22.**), and the control sample (C14) was separated from the added-samples (R14, E14, and AC14). Meatballs with rowanberry pomace ingredients showed some differences in their chemical profile, while 2%-R and 1%-E samples were very close to each other. In contrast, 2%-AC showed a more characteristic chemical profile. 1%-E ingredient was the most active rowanberry ingredient against the accumulation of aldehydes and ketones, compared with the control after 14 days of the storage period. Our results showed that linoleic acid derivatives showed an overall down-accumulation in the control sample, which indicates a potentially higher lipid peroxidation that was maintained by the addition of rowanberry pomace ingredients. An effect of the storage time was particularly evident when considering samples 2%-AC and 1%-E.

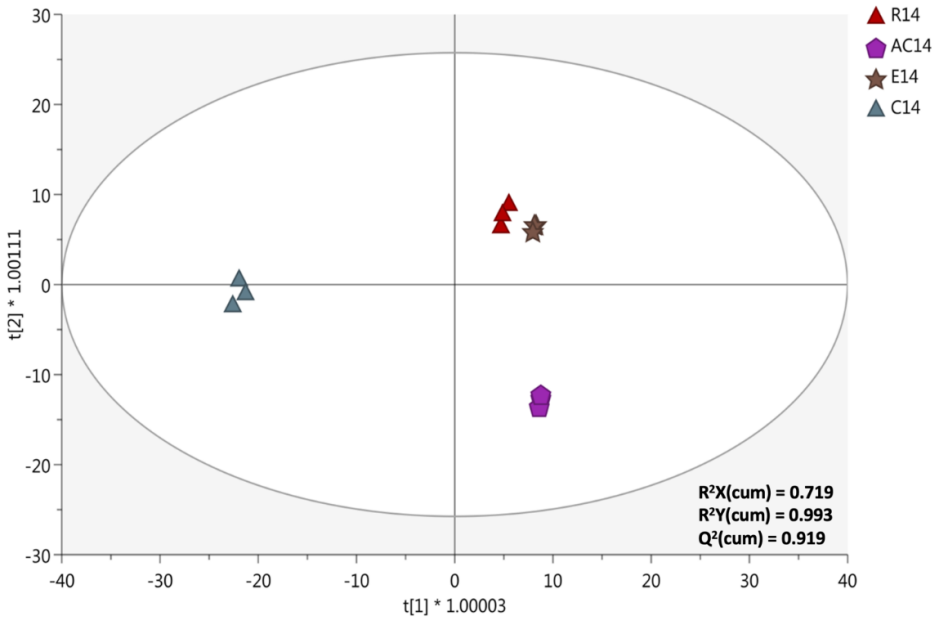

Fig. 3.22. OPLS-DA analysis considering the chemical profile of different meatballs prepared with rowanberry functional ingredients (AC, E, and R) vs. the control (C) at 14 days of the storage time period

This analysis identified the main phenolic compound in meatballs with rowanberry ingredients as chlorogenic acid and isoquercitrin, which has proven the presence of antioxidant activity against free radicals (Sarv[111]). It was also hypothesized earlier in this work that chlorogenic acid may exert some effect on the quality of meatballs, especially regarding the pH-level. Meatballs containing rowanberry pomace ingredients had a significant up-accumulation of these compounds at the end of the storage time, which supports the hypothesis of rowanberry having a greater protection against lipid oxidation processes.

3.2.8. Application possibilities of plant-based ingredients to meat products

The convenience food sector is one of the fastest growing sectors of the food industry. Due to the growing consumer preferences for ‘natural’ food, there is a tendency to use raw materials of the plant origin. One of such raw materials is industrial hemp (*Cannabis sativa* L.) and hemp-processing by-products serving as an important source of protein replacing animal-based proteins due to their amino acid composition. A by-product of the pressing process of hemp seeds is hempseed cake. Currently, their use for food purposes is rather scarce, but the high nutritional value encourages to search for ways to use it in the food industry for the excellent nutraceutical value, digestibility, and physicochemical properties. Hempseed proteins are denoted by good nutritional value, as its protein contains all the

essential amino acids in a balanced ratio. Additionally, hempseed proteins have lower allergenicity than most other plant proteins (Chen *et al.*[93]). The current research has demonstrated the successful use of hempseed press-cake ingredients in the production of pork patties with an addition of 2%. Hempseed cake as a protein-rich ingredient had an effect on grilling losses, especially defatted hemp (DH), while decreasing it significantly (14.34%) compared to the control sample (24.2%). High scores in the sensory assessment for appearance, colour and taste indicate that such protein-rich ingredients are acceptable for consumers and may be used in meat products.

One of the most significant trends in the food science and technology is the upcycling of agri-food processing side-streams for the recovery of high nutritional value compounds and the production of health-beneficial components for diverse uses, including meat products. Actually, two terms are used in the utilization (or valorization) of side-streams (by-products), namely, upcycling and recycling. Recycling is considered as broken-down (waste or by-product) into another product or material, often one of a lesser quality; while upcycling takes a waste (by-product) in its current state and repurposes it into something of a higher value. The results obtained from this research showed that upcycling of various berry pomace ingredients at concentrations from 1% to 5% gave good results and can be used as antioxidants and the overall quality enhancers in meat products. In addition, environmentally friendly extraction methods were used to obtain different plant-based ingredients. Some of the plant-based extracts as valuable products were defatted by CO_2 extraction, whereas some were produced by using EtOH and H_2O as solvents. By using *in vitro* testing, the obtained extracts showed good antioxidant activity.

Fruit processing by-products are rich in polyphenolic antioxidants. Small fruits are therefore particularly interesting for upcycling into more valuable materials. For that, the use of novel green extraction technologies has been tested for the valorization of berry pomace of various small fruits. Blackcurrant seeds after supercritical fluid CO_2 extraction (BC-ASC) showed a stronger antioxidant activity when using TPC, ORAC and $ABTS^{\bullet+}$ assays compared to raw dried seeds (BC-RS) or seeds after EtOH/water extraction (BC-AE). TPC, ORAC and $ABTS^{\bullet+}$ values obtained for BC-ASC were 62.09 mg GA/g, 141.31 TE mg/Trolox/g and 15.62 TE mg Trolox/g, respectively. It can be explained by the removal of lipophilic compounds and oil, which led to an increased proportion of polyphenolic antioxidants.

BC dried and milled raw skins showed strong antioxidant activity in TPC and ORAC assays, while defatted skins with $scCO_2$ had a strongest activity when using $ABTS^{\bullet+}$ (305.70 TE mg Trolox/g) followed by skins after all extractions ($ABTS^{\bullet+}$ 262.41 TE mg Trolox/g). The lowest antioxidant activity in all assays compared to other skin ingredients was detected in a combined extract of defatted residues consecutively isolated with EtOH and H_2O (HOE), which also indicated the highest TBARS values during lipid oxidation. These results indicate that BC skin ingredients can be perceived as promising natural antioxidants which could be effectively used in meat products so that to prevent lipid oxidation during storage.

BC seed ingredients affected the chemical composition, the cooking loss, and the colour of pork meatballs. A significant effect was noticed for the fibre content – the higher was the inclusion level of BC seed ingredients, the higher was the fibre content in pork meatballs. The highest dietary fibre content was in samples BC-ASC, which confirmed that, after CO_2 extraction, the percentage of dietary fibre increases. In addition, meatballs with 5% of BC-ASC showed the lowest cooking loss (19.77%) compared to the control sample (26.79%); they had a high protein and fibre content (22.85 g/100g and 2.128%, respectively). The inclusion of 5% of various BC ingredients had an effect on the cooking losses, as they were the lowest compared to the control sample. The preliminary sensory evaluation of meatballs with different concentrations of BC seed ingredients was overall positive by the assessors. To conclude, BC seed ingredients at concentrations of 1, 3 and 5% are acceptable for consumers even despite its colour, and, as it is from known sources, it does not lower the consumers' expectations. Blackcurrant seeds after scCO_2 extraction (full defatting) were indicated as the most effective ingredient in reducing the amount of oxidation-related derivatives. BC seed ingredients seem to be promising antioxidants and fibre-rich materials for the application in meat products.

Lipid oxidation contributes to the changes in the meat colour, taste, flavour, and nutritional value. To reduce the occurrence of such changes, the addition of natural antioxidants is applied. Sweet grass extract completely inhibited the oxidation process of pork patties during 21 days of storage compared to the control sample, or especially in patties with raw hemp. 5,8-dihydroxycoumarin found in sweet grass was found to have a strong antioxidant activity, and therefore it was found to inhibit the oxidation processes. The TBARS values for SG remained in the range of 0.175 mg/MDA/kg, while, for the control sample, it increased from 0.420 to 0.540 mg/MDA/kg, and, in samples with raw hemp, it went up from 0.181 to 0.364 mg/MDA/kg. Despite the positive effect of SG on the lipid oxidation, its use had some negative effects on the sensory characteristics in raw and grilled pork patties due to its bitter taste and the unusual dark-green colour. The scores for taste were the lowest in samples with RHSG and SG, which were below 3.75 and 4, *i.e.*, which were below satisfactory. On the other hand, what concerns juiciness, the samples with SG got higher ratings (5.88) compared to the control sample (below 5).

The high effectiveness of blackcurrant seeds after supercritical fluid CO_2 extraction indicate the most effective ingredient in reducing the number of oxidation-related derivatives. It also had a high protein content, and also had an effect on the cooking loss while decreasing it in a dose-dependent manner. BC seed ingredients seem to be promising natural ingredients and fibre-rich materials to be used in meat products also positively affecting the nutritional value.

Research on the valorization of rowanberry pomace is rather scarce; therefore, the currently obtained data is rather valuable. Rowanberry pomace EtOH/water extract was found to be the most successful at preventing unpleasant flavours on the last day of storage. The decrease in lipid oxidation was observed when adding rowanberry pomace ingredients, which was indicated by reduced 7-dodecenal and 2,4-heptadienal contents. Also, the fibre-rich ingredient R (extraction residue) had a positive effect on the cooking loss of meatballs being the lowest (20.17%) compared

to the control sample (23.33%). Despite some positive effects of rowanberry pomace ingredients on the quality characteristics, there are some sensory limitations of applying it to meat products – as more than 2% of rowanberry pomace ingredients is not acceptable for consumers due to its astringent taste.

Furthermore, it is possible that the addition of bioactive berry and other plant-based phytochemicals into meat products can enhance their overall quality, oxidative stability, and perhaps even provide some health-related benefits. Tested plant-based ingredients have potential to be added at concentrations between 1–5% into minced meat products. They are popular in Europe, and their quality characteristics and chemical profile are influenced positively. The results also indicate that plant-based ingredients are denoted by prospects to be used at the industrial level (with some pilot-scale studies), encouraged by the fact that the used plant-based ingredients are cheap and readily available; they also have the food-grade (GRAS) status; from the scientific point of view, they ensure scientific novelty. In addition, meat producers are willing to replace expensive meat with cheap plant-based ingredients and enrich meat products with health-beneficial phytochemicals. Upcycling of agri-food processing side-streams into high-nutritional value ingredients to be used in meat products has evolved into an important trend in the food science and technology. Further studies should focus on the effectiveness of other quality-related characteristics and upcycling of other agri-food processing side-streams.

CONCLUSIONS

1. The selected plant-origin ingredients showed good antioxidant activity and were sensorically acceptable to be used in cooked meat products.

1.1. Sweet grass (SG) ethanolic extract as a natural antioxidant showed a strong antioxidant activity, especially in the ORAC assay (30.65 ± 1.64 mmol TE/g dw). Moreover, TPC observed in SG was 99.04 mg GA/g dw, $DPPH^{\bullet}$ 300.3 mg TE/g dw, and $ABST^{\bullet+}$ 692 mg TE/g dw.

1.2. Blackcurrant seeds after supercritical fluid CO_2 extraction (BC-ASC) showed a stronger antioxidant activity when using TPC, ORAC and $ABTS^{\bullet+}$ assays comparing to raw dried seeds (BS-RS) or seeds after EtOH/water extraction (BC-AE). TPC, ORAC and $ABTS^{\bullet+}$ values obtained for BC-ASC were 62.09 mg GA/g, 141.31 TE mg/Trolox/g and 15.62 TE mg Trolox/g, respectively. It can be explained by the removal of lipophilic compounds and oil leading to the increased proportion of polyphenolic antioxidants. BC-AE had higher $ABTS^{\bullet+}$ values (109.63 mg GA/g), which, for BC-RS, were 74.12 mg GA/g.

1.3. In terms of the antioxidant activity and phenolic content, three rowanberry pomace powders of cultivars Likernaja, Solnechnaja and wild rowanberry were selected. Likernaja was denoted by the highest $DPPH^{\bullet}$ value (527.55 μM TE/g dw), Solnechnaja contained a very high ORAC value (146.6 μM TE/g dw), whereas wild rowanberry had a high $DPPH^{\bullet}$ value (358.6 μM TE/g dw) and an ORAC value of 313.2 μM TE/g dw. What concerns the preliminary TPC analyses of those 3 ingredients, the TPC value of ingredient E (EtOH/water extract of AC) was almost five-fold higher compared to the TPC value of AC (defatted with supercritical CO_2) and 17 times higher than the value of R (extraction residue).

1.4. The current research demonstrated that the selected plant origin ingredients may be considered as promising antioxidants to be used in cooked meat products at concentrations 1–5%, which improved their quality characteristics. In the course of the sensory evaluation, specific acceptable nutty odour for patties with hemp ingredients was described, which also had a positive effect on juiciness, especially in defatted by supercritical CO_2 extraction hempseed cake compared to the control sample. The high scores for appearance, colour and taste indicate that such protein-rich ingredients are acceptable for consumers and may be used in meat products. It was agreed on the basis of the sensory evaluation that more than 2% of rowanberry pomace ingredients is not acceptable for consumers due to its astringent taste. According to the preliminary sensory assessment, meatballs with different concentrations of BC seed ingredients were positively evaluated by the assessors.

In the case of sweet grass extract in pork patties, even at very low concentrations (0.5%), it affected most prominently the sensory parameters of raw and grilled ones, while it received the lowest scores from the

panellists for all assessed parameters due to the unusual green colour and bitter taste.

2. Selected plant origin ingredients influenced quality characteristics of cooked meat products and may also be promising ingredients to enrich meat products with health-beneficial phytochemicals.

2.1. BC seed ingredients seem to be promising antioxidants and fibre-rich materials for the application in meat products, which may increase their nutritional quality related to health benefits. They were found to affect the chemical composition, the cooking loss, and the colour of pork meatballs. A significant effect was noticed regarding the fibre content – the higher is the inclusion level of BC seed ingredients, the higher is the fibre content in pork meatballs. The highest dietary fibre content was determined in samples BC-ASC (blackcurrant seeds after supercritical fluid CO_2 extraction), which confirmed that, after CO_2 extraction, the percentage of dietary fibre increases. In addition, meatballs with 5% of BC-ASC showed the lowest cooking loss while having a high protein and fibre content (22.85 g/100g and 2.128%, respectively). Inclusion of 5% of various BC ingredients had an effect on cooking losses, and it was the lowest compared to the control sample. Meatballs with fibre-rich ingredient 2%-R (extraction residue of rowanberry pomace) had the lowest cooking loss (20.17%) compared to the control sample (23.33%). From the current research, the lowest grilling loss was observed in patties with defatted by supercritical CO_2 extraction hempseed cake (14.34%) compared to the control sample (24.2%), which can be explained by the high protein content (51.7%) in this ingredient, and therefore these samples were denoted by good water-holding capacity. This knowledge may be useful for producers, as proteins are essential components in food; they also help to improve, for instance, the water-binding capacity, and, therefore, reduce production losses.

2.2. All the used plant-origin ingredients were found to influence the colour characteristics by decreasing the lightness (L^*) of cooked meat products, with a progressive increase in redness (a^*) in samples with BC seed and rowanberry pomace ingredients with a great probability of the content of anthocyanin pigments.

3. An effect of plant-origin ingredients on some meat quality characteristics during the storage was detected.

3.1. Sweet grass showed a remarkable antioxidant activity, and its extract effectively stabilized the formation of TBARS in pork patties during the 21 days of storage compared to the control sample, or especially together with raw hemp. TBARS assay values for SG remained in the range of 0.160–0.186 mg/MDA/kg, while, for the control sample, the values increased from 0.420 to 0.540 mg/MDA/kg, and, in samples with raw hemp, the values went up from 0.181 to 0.364 mg/MDA/kg. The highest TBARS assay values were also detected in samples with raw hempseed cake after 15 and 21 days of storage, which supported the

hypothesis that unsaturated oil residues in raw hempseed press-cake may foster the formation of oxidation products.

3.2. During 5 days of storage at 4 °C, the pH of meatballs with 1%-E was significantly lower compared to the control sample, which may be explained by the presence of chlorogenic acids.

3.3. According to unsupervised statistics, the impact of the storage time on the chemical profile of meatball samples was also detected. Among the meatballs with 1% of EtOH/water extract of AC, the most active impact was noted for the rowanberry ingredient against the accumulation of aldehydes and ketones compared with the control sample at 14 days of storage time. Also, during 14 days of storage, rowanberry pomace ingredients inhibited the development of unpleasant flavours caused by carbonyl compounds. The concentration of linoleic acid derivatives decreased only in the control sample. These findings confirm the hypothesis that rowanberry pomace ingredients can be used as functional ingredients.

4. BC seed and rowanberry pomace ingredients demonstrated the potential effect on modifying the metabolomic profile of cooked meatballs.

4.1. According to unsupervised statistics, the most efficient treatments capable of significantly changing the chemical profile of meatballs were those based on the addition of high levels (3–5%) of BC seeds before and after supercritical fluid CO_2 extractions. Blackcurrant seeds after $scCO_2$ extraction (full defatting) were pinpointed as the most effective ingredient in reducing the number of oxidation-related derivatives. Rowanberry pomace EtOH/water extract (1%-E) was the most effective ingredient against the development of unpleasant flavours caused by carbonyl compounds at day 14. The decrease in lipid oxidation, indicated by reduced 7-dodecenal and 2,4-heptadienal contents, was observed when adding rowanberry pomace ingredients containing chlorogenic acid as an antioxidant. The up-accumulation of these compounds may be associated with the effect of rowanberry pomace ingredients on lipid oxidation. These results demonstrated the potential effect of rowanberry pomace ingredients on modifying the metabolomic profile of meat.

4.2. Chlorogenic acid and isoquercetin as the major phenolic compounds associated with rowanberry ingredients were the most prevalent compounds in meatballs. The metabolomic dataset revealed a distribution of phenolic compounds, likely from BC seeds: flavonoids, with a great abundance of anthocyanins, flavones, flavonols, and other compounds, phenolic acids (*e.g.*, gallic, chlorogenic, and sinapic acids), stilbenes, lignans, and tyrosol-derivatives.

4.3. According to untargeted metabolomic findings, both the percentage of inclusion and the extraction method used are crucial factors which need to be carefully evaluated so that to improve the overall quality of cooked pork meat products.

SANTRAUKA

Mėsa yra svarbus maisto produktas, aprūpinantis žmonių organizmą vertingomis mitybai medžiagomis – didelės biologinės vertės baltymais, mineralinėmis medžiagomis, ypač biologiškai pasisavinama geležimi, vitaminais ir kitais būtinais elementais. Gyvūninės kilmės maisto produktų paklausa visame pasaulyje didėja (iki 2030 m. 14 %), nors per daug valgyti mėsos nerekomenduojama, o požiūris į mėsą ir jos poveikį žmonių sveikatai pastaruoju metu tapo nelabai teigiamas. Vartotojų preferencijos keitėsi, ypač natūralesnių ingredientų paklausos didėjimo kryptimi. Tai skatina mėsos pramonę kurti naujus ingredientus ir žaliavų perdirbimo metodus. Maltos mėsos gaminiai yra labai jautrūs mikrobiologiniams ir oksidacijos procesams, sukeliantiems skirtingus kokybės defektus. Sandėliuojant ir perdirbant mėsą bei mėsos produktus vyksta įvairios (bio)cheminės reakcijos, kurios gali neigiamai paveikti kokybę. Tos nepageidaujamos reakcijos gali būti kontroliuojamos įvairiomis fizinėmis priemonėmis arba naudojant augalinės kilmės ingredientus, kuriuose gausu antioksidantų. Per pastaruosius kelerius metus labai išpopuliarėjo įvairių augalinės kilmės ingredientų pritaikymo mėsos gaminiuose bandymai. Nemažai tyrimų atlikta su medžiagomis, kurios išskiriamos iš šalutinių vaisių ir uogų perdirbimo produktų, dažnai pasižyminčių stipriu bioaktyvumu. Kita svarbi maisto mokslo ir technologijų tendencija yra žemės ūkio ir maisto gamybos šalutinių srautų perdirbimas, siekiant išskirti didelės mitybinės vertės junginius, sukuriant sveikatai naudingus komponentus, kurie taip pat gali būti pritaikyti mėsos gaminiuose. Pridėjus augalinės kilmės medžiagų, kuriose gausu bioaktyvių junginių, galima sulėtinti arba atitolinti oksidacijos procesus, padidinti galiojimo laiką ir / ar kontroliuoti mikroorganizmų dauginimąsi. Kadangi mėsos gamyba taip pat susijusi su poveikiu aplinkai, pastebima tendencija kurti mėsos produktus iš dalies arba visiškai pakeičiant brangius gyvūninius baltymus augalinės kilmės ir netradiciniais ingredientais, kartu gerinant bendrąją gaminių kokybę arba didinant jų naudą sveikatai. ir maistinę vertę. Aplinkosaugos klausimai yra sprendžiami kuriant efektyvias žemės ūkio ir maisto produktų šalutinių produktų perdirbimo technologijas. Vadinamieji žaliosios gavybos metodai, kurie buvo paskatinti išaugusio visuomenės susidomėjimo produktais be chemikalų, įgalino sukurti natūralius ingredientus, kuriuose gausu fitocheminių medžiagų, išskirtų iš vaisių ir daržovių atliekų bei šalutinių perdirbimo produktų. Pastaruosius kelerius metus įvairios uogų išspaudos buvo išbandytos naudojant efektyvią ir ekologišką nuoseklaus ekstrahavimo platformą. Tokių inovatyvių technologijų, kaip ekstrahavimas virškriziniu CO_2 ir suslėgtais skysčiais, pritaikymas biologiškai aktyvių sudedamųjų dalių išskyrimui gali būti laikomas labai perspektyvia žemės ūkio maisto atliekų bei šalutinių perdirbimo produktų vertės didinimo tendencija.

Tyrimai parodė, kad biologinio smulkių vaisių išspaudų rafinavimo būdu galima sukurti aukštos maistinės vertės ingredientus, kurie gali būti sėkmingai pritaikomi ir mėsos gaminiuose. Pavyzdžiui, yra gerai ištirta, kad stumbražolės (*Hierochloe odorata* (L.) P. Beauv.) ekstraktas pasižymi stipriu antioksidaciniu

potencialu. Pastaruoju metu labai išaugo susidomėjimas pluoštinių kanapių (*Cannabis sativa* L.) sėklomis ne tik kaip aukštos kokybės aliejaus šaltiniu, bet ir kaip baltyminiu ingredientu. Šermukšnių (*Sorbus aucuparia* L.) ir juodųjų serbentų (*Ribes nigrum* L.) išspaudose gausu polifenolinių antioksidantų. Aukščiau išvardinti perspektyvūs baltymų šaltiniai ir natūralūs antioksidantai anksčiau nebuvo tirti mėsos produktuose: šiame darbe naudojant ekologiškus tirpiklius ir ekstrahavimo technologijas jie pirmą kartą išbandyti mėsos gaminiuose.

Šio darbo tikslas buvo ištirti skirtingus augalinės kilmės ingredientus bei jų ekstraktus mėsos gaminiuose ir įvertinti jų poveikį bendrosioms mėsos kokybės savybėms, taip pat įvairių cheminių junginių sudėčiai, įvertinant ją netikslinės metabolomikos metodu. Buvo iškelta hipotezė, kad šie augalinės kilmės ingredientai galėtų pagerinti bendrąją mėsos produktų kokybę, ypač slopinant lipidų oksidacijos procesus laikymo metu. Tikslui pasiekti buvo atrinkti ir apibūdinti perspektyvūs augalinės kilmės ingredientai – kanapių sėklų išspaudos, stumbražolės (SG) ekstraktas, juodųjų serbentų (BC) ir šermukšnių (RB) išspaudos bei ekstraktai, pasižymintys stipriu antioksidaciniu poveikiu. Augalinės medžiagos buvo išskiriamos taikant įvairius ekologiškos ekstrakcijos būdus, dozuojamos į mėsos produktus skirtingomis koncentracijomis ir įvertinta jų įtaka mėsos gaminių kokybės rodikliams – spalvos parametrams, pH vertei, vandens aktyvumui (a_w), cheminei sudėčiai, virimo nuostoliams, oksidacijai, juslinėms savybėms ir įvairių cheminių junginių sudėčiai, įvertinant ją netikslinės metabolomikos būdu.

Šiame darbe buvo naudojami ekologiški ir inovatyvūs ekstrahavimo būdai. Stumbražolės etanolinis ekstraktas, kaip natūralus antioksidantas, pasižymėjo stipriu antioksidaciniu aktyvumu, ypač nustatytu ORAC metodu (30,65 ± 1,64 mmol TE/g dw). Be to, bendrasis fenolinių junginių kiekis (TPC) buvo 99,04 mg GA/g s.m., $DPPH^{\bullet}$ sujungimo geba 300,3 mg TE/g s.m. ir $ABTS^{\bullet+}$ sujungimo geba 692 mg TE/g s.m. Pagal TPC, ORAC ir $ABTS^{\bullet+}$ tyrimų rezultatus juodųjų serbentų sėklų antioksidacinis aktyvumas po ekstrahavimo virškriziniu CO_2 (BC-ASC) buvo stipresnis negu neapdorotų išdžiovintų sėklų (BS-RS) bei sėklų liekanų po ekstrahavimo EtOH/vandens (BC-AE) mišiniu. BC-ASC gautos TPC, ORAC ir $ABTS^{\bullet+}$ reikšmės buvo atitinkamai 62,09 mg GA/g, 141,31 TE mg/Trolox/g ir 15,62 TE mg Trolox/g. Tai galima paaiškinti lipofilinių junginių ir aliejaus pašalinimu, dėl kurio padidėja polifenolinių antioksidantų dalis. BC-AE $ABTS^{\bullet+}$ vertės buvo didesnės (109,63 mg GA/g), o BC-RS – 74,12 mg GA/g. Pagal antioksidacinį aktyvumą atrinkti trijų veislių – Likernaja, Solnechnaja ir laukinių šermukšnių išspaudų milteliai. Likernaja veislėse nustatyta didžiausia $DPPH^{\bullet}$ reikšmė (527,55 µM TE/g s.m.), Solnechnaja – ORAC vertė (146,6 µM TE/g s.m.) ir laukinių šermukšnių mėginiui - $DPPH^{\bullet}$ vertė (358,6 µM TE/g s.m.) ir ORAC vertė 313,2 µM TE/g dw.

Labai reikšmingi BC sėklų ekstraktų sudėties skirtumai nebuvo nustatyti, tačiau baltymų ir maistinių skaidulų kiekis ekstrahavimo liekanose buvo šiek tiek didesnis, palyginti su neekstrahuota žaliava. Tai galima paaiškinti tirpių lipofiliškų junginių pašalinimu ekstrahuojant virškriziniu CO_2. Baltymų kiekis BC sėklose prieš ekstrakciją CO_2 buvo 20,17 %, skaidulų kiekis 17,28 %, o baltymų kiekis BC sėklose po ekstrahavimo hidroetanoliniu tirpikliu ir virškriziniu CO_2 buvo

atitinkamai 22,53 % ir 21,15 %; skaidulų kiekis atitinkamai 19,74 % ir 17,4 %. Baltymų kiekis kanapių sėklose buvo 36,6 %, o išdžiovintose mechaniškai presuotose kanapių išspaudose (RH, neekstrahuotos kanapės) – 51,7 %. Neekstrahuotose išspaudose (DH), iš kurių buvo pašalinti riebalai virškriziniu CO_2, skaidulų kiekis buvo atitinkamai 21 ir 26.1%. Žaliavinių kanapių sėklų išspaudų ekstrakcija virškriziniu CO_2 sumažino riebalų kiekį nuo13,3% (RH) iki 1.4% (DH). Todėl galima pagrįstai tikėtis, kad pašalinus aliejaus likučius, turėtų būti sumažinta gauto produkto oksidacijos rizika ir su ja susijęs neigiamas poveikis.

Nedidelio kiekio kanapių sėklų išspaudų ir stumbražolės ekstrakto pridėjimas į keptus kiaulienos gaminius arba šermukšnių išspaudų į kotletus neturėjo jokios reikšmingos įtakos bendriesiems cheminės sudėties rodikliams. Tačiau riebalų kiekis sumažėjo – ypač mėsos kukuliuose su daug skaidulų turinčiais ingredientais AC (15,20 %) ir R (17 %), palyginti su kontroliniu mėginiu (21,05 %). Bet BC sėklų ingredientų pridėjimas turėjo įtakos kiaulienos kotletų cheminei sudėčiai. Beveik visuose gaminiuose buvo daugiau baltymų, palyginti su kontroliniu mėginiu. Pagal gautus rezultatus, kuo didesnė BC sėklų ingredientų dozė, tuo didesnis skaidulų kiekis gaminyje.

Visi ingredientai turėjo įtakos virimo nuostoliams ir spalvos savybėms. Kepimo nuostoliai buvo išmatuoti atšaldžius produktą iki kambario temperatūros ir pasvėrus mėsos gaminius prieš ir po terminio apdorojimo. Mažiausi kepimo nuostoliai buvo nustatyti gaminiuose, į kuriuos buvo pridėta kanapių sėklų išspaudų (14,34 %) iš kurių pašalinti riebalai, palyginti su kontroliniu mėginiu (24,2 %), ir tai galima būtų paaiškinti dideliu baltymų kiekiu tokiose išspaudose (51,7 %). Mažesni kepimo nuostoliai taip pat buvo nustatyti bandiniuose su neapdorotomis kanapių išspaudomis. Pridėjus 5 % skirtingų BC ingredientų, terminio apdorojimo nuostoliai buvo mažiausi, palyginti su kontroliniu gaminiu; mažiausi nuostoliai nustatyti kotletuose su 5 % su 5 % juodųjų serbentų sėklų, iš kurių virškriziniu CO_2 pašalinti riebalai, priedu, kuriame taip pat yra daug baltymų ir skaidulų, atitinkamai 22,85g/100g ir 2,128 %. Mėsos kukuliai, į kuriuos pridėta 2% skaidulų turinčio šermukšnio išspaudų liekanų po ekstrakcijos, patyrė mažiausius virimo nuostolius (20,17 %), palyginti su kontroliniu mėginiu (23,33 %) arba su EtOH/vandens ekstraktu (26,23 %).

Poveikis spalvos charakteristikoms ir pokyčiams mėsos gaminių laikymo metu buvo įvertintas CIE (Tarptautinės apšvietimo komisijos) Lab sistema, kur *L** reiškia šviesumą, *a** – raudonumą ir *b** – geltonumą. SG ekstraktas smarkiai sumažino kiaulienos paplotėlių šviesumą dėl savo tamsiai žalios spalvos. Kanapių sėklų išspaudos, kaip šviesiai žalios spalvos ingredientai, taip pat sumažino *L** vertes, palyginti su kontroliniu gaminiu. Tokius pokyčius taip pat nustatė juslinės analizės komisijos nariai. BC sėklų ingredientų pridėjimas turėjo įtakos kiaulienos kotletų spalvos parametrams (*L**, *a** ir *b**) tikriausiai dėl juose esančių antocianino pigmentų. Buvo akivaizdu, kad didinant BC sėklų dozę, mėsos gaminių spalva tapo tamsesnė (mažesnė *L** vertė); *a** vertės padidėjimas buvo pastebėtas visuose mėginiuose su BC sėklų ingredientais, palyginti su kontroliniu gaminiu. O *b** vertės didinant BC sėklų kiekį mėsos gaminiuose mažėjo. Mėsos kukulių su šermukšnių išspaudų ingredientais *L** vertė smarkiai sumažėjo dėl šermukšniuose esančių

antocianinų. Vertinant kotletus su BC sėklų sudedamosiomis dalimis, verta pažymėti, kad išspaudų ekstraktai sumažino geltonumą, ypač pridėjus 1%daug biologiškai aktyvių komponentų turinčio EtOH/vandens ekstrakto (E) ir 2 % AC.

pH, kaip svarbus mėsos ir mėsos produktų kokybės parametras, siejamas su kitais kokybės rodikliais, pvz., mikroorganizmų augimas yra optimalus, kai pH 7,0. Keptų kiaulienos gaminių su apdorotomis kanapių sėklų išspaudomis ir stumbražolės ekstraktu pH laikymo laikotarpiu buvo 6,1–6,3. BC sėklų ingredientai turėjo įtakos kotletų pH. Mažiausias pH po 3 ir 6 dienų laikymo buvo nustatytas kontrolinio gaminio (<6); toks pH yra palankus oksidacijai. Kituose mėginiuose pH vertės su BC sėklų ingredientais praktiškai nesikeitė. Panašius rezultatus publikavo ir kiti autoriai. Per 5 laikymo dienas, pridėjus 1% šermukšnių išspaudų EtOH/vandens ekstrakto, pH buvo daug mažesnis, palyginti su kontroliniu gaminiu; tai gali būti paaiškinta organinių rūgščių, tokių kaip chlorogeno rūgštis, buvimas ekstrakte.

Kartu su pH vandens aktyvumas (a_w) yra svarbus veiksnys produkto stabilumui sandėliavimo metu. Vandens aktyvumas nėra tas pats, kas drėgmės kiekis: kuo mažesnis aw, tuo ilgesnis gaminio galiojimo laikas. Šviežios mėsos a_w yra labai didelis, 0,99. Mėsą perdirbant į mėsos produktus, produkto vandens aktyvumas mažėja, priklausomai nuo įvairių veiksnių (produkto druskos kiekio, pH, higienos perdirbimo metu ir kt.). Kai a_w mažesnis nei 0,75, mikroorganizmai neauga, o daugumos jų vystymasis sulėtėja kai aw sumažinamas iki 0,91. Kiaulienos gaminių su kanapių sėklų ingredientais ir stumbražolės ekstraktu aw vertės per 21 laikymo dieną buvo 0,950–0,963; rodiklis buvo didesnis gaminiuose su kanapių sėklų išspaudų ir stumbražolės ekstrakto mišiniu. Mėsos kukulių su BC sėklų ingredientais aw vertės 6 laikymo dienų periodu buvo 0,978–0,984, t. y. gana stabilios tieks skirtinguose gaminiuose, tiek skirtingu laikymo metu. Naudojant šermukšnių išspaudų sudedamąsias dalis, išmatuotos kotletų aw vertės buvo 0,974–0,987. Nedidelis sumažėjimas (1,3 %) buvo nustatytas po 5 laikymo dienų mėsos kukuliuose, pagamintuose su išspaudų liekana po ekstrahavimo – nuo 0,987 iki 0,974. Toks sumažėjimas neturi reikšmingos įtakos kotletų galiojimo laikui. Apibendrinant galima pasakyti, kad pridėjus augalinių ingredientų ir lyginant su kontroliniu gaminiu, nustatytas tik nedidelis poveikis a_w vertėms.

TBARS (su tiobarbitūro rūgštimi reaguojančių medžiagų kiekis) metodas buvo naudojamas norint greitai įvertinti lipidų oksidacijąį . SG ekstraktas pasižymėjo puikiu antioksidaciniu aktyvumu, efektyviai stabilizuodamas TBARS susidarymą kiaulienos gaminiuose laikant juos 21 dieną ir lyginant su kontroliniu gaminiu arba su gaminiu, į kurį buvo pridėta žaliavinių kanapių išspaudų. Tai gali būti paaiškinama tuo, kad stumbražolės ekstrakte esantis 5,8-dihidroksikumarinas yra labai stiprus antioksidantas: gaminių su SG TBARS reikšmės buvo iki 0,175 mg/MDA/kg, kontrolinių gaminių padidėjo nuo 0,420 iki 0,540 mg/MDA/kg, o gaminių su neapdorotomis kanapių išspaudomis nuo 0,181 iki 0,364 mg/MDA/kg. Didžiausios TBARS vertės buvo nustatytos gaminiuose su neapdorotomis kanapių sėklų išspaudomis po 15 ir 21 dienų laikymo, o tai patvirtina hipotezę, kad nesočiųjų riebalų likučiai kanapių sėklų išspaudose, iš kurių pašalinti riebalai, gali paskatinti oksidacijos produktų susidarymą. Nustatyta, kad BC sėklų ingredientai yra

perspektyvūs antioksidantai ir daug skaidulų turinčios medžiagos, kurios turi didelę įtaką ląstelienos kiekiui mėsos gaminiuose; daugiausia skaidulinių medžiagų nustatyta kotletuose su 5 % sėklų išspaudų po ekstrahavimo virškriziniu CO_2 – 2,1 %. Tai paaiškinama tuo, kad po CO_2 ekstrahavimo maistinių skaidulų procentinis kiekis išspaudose proporcingai padidėja. Veiksmingiausias ingredientas mažinant su oksidacija susijusių junginių kiekį buvo BC sėklos po ekstrahavimo virškriziniu CO_2, kurios taip pat pasižymėjo stipriu antioksidaciniu aktyvumu, nustatytu TPC, ORAC ir $ABTS^{\bullet+}$ metodais. Šermukšnių išspaudų ingredientas, kuriame nustatyta antioksidanto chlorogeno rūgšties, sprendžiant pagal sumažėjusį 7-dodecenalio ir 2,4-heptadienalio kiekį, taip pat slopino lipidų oksidacijos procesus.

Kiaulienos gaminių ir kotletų juslinės savybės buvo vertinamos pagal jų išvaizdą, spalvą, kvapą, skonį ir sultingumą taikant 9-balų hedoninę skalę, kuri plačiai naudojama kaip palyginamasis metodas išbandant naujus ingredientus. SG ekstraktas turėjo didžiausią įtaką kiaulienos paplotėlių jusliniams rodikliams, todėl gaminiai su SG ir RHSG (džiovintų presuotų kanapių sėklų išspaudų ir stumbražolės ekstrakto mišinys) gavo žemiausius komisijos narių balus pagal visus vertintus parametrus, išskyrus sultingumą (mažiau balų skirta kontroliniam gaminiui). Kontrolinio gaminio išvaizda ir spalva buvo įvertinti aukščiausiais balais, ir tai koreliuoja su spektrofotometrinio įvertinimo rezultatais, pagal kuriuos tamsiai žalias SG ekstraktas šviesumą sumažino 16–20 %. Keptų paplotėlių juslinio vertinimo metu vertintojai pažymėjo nebūdingą tokiems gaminiams žalią spalvą. Gaminių su SG kvapas taip pat buvo įvertintas mažiausiais balais, vidutiniškai 5,3. Gaminiams su RHSG ir SG už skonį skirti balai buvo mažesni nei 3,75 ir 4, t. y. nepatenkinami. Kita vertus, už sultingumą mėginiai su SG gavo aukštesnius įvertinimus (5,88), palyginti su kontroliniu gaminiu (<5). Keptų paplotėlių su kanapių sėklų ingredientais rezultatai buvo gana panašūs – visos įvertintos charakteristikos buvo nuo 6 iki 7. Mėginiuose su kanapių ingredientais buvo įvardinti specifiniai, tačiau priimtini riešutų kvapo tonai. Remiantis atliktais tyrimais, gaminiai su DH ir RH gavo gana aukštus balus už išvaizdą, spalvą ir skonį, o tai rodo, kad baltymingi kanapių sėklų išspaudų ingredientai yra priimtini vartotojams ir gali būti toliau naudojami kuriant mėsos gaminius.

Augalinės kilmės ingredientų, tokių kaip konservantai ar antioksidantai, naudojimas maisto produktuose gali būti ribotas dėl jų juslinių savybių. Preliminariai juslinio vertinimo metu buvo ištirtos 1–5 % šermukšnių išspaudų ingredientų koncentracijos kotletuose ir parinkta kiekvienai grupei priimtiniausia dozė. Preliminarus vertinimas yra dažniausiai taikoma praktika, siekiant nustatyti vartotojams priimtiną formulę. Daugiausia balų už skonį (7,5) vertintojai skyrė gaminiui su 1 % išspaudų liekanos po ekstrahavimo; ši dozė taip pat priimtiniausia vertinant kvapą. Gaminiai su daugiau nei 1 % EtOH/vandens ekstrakto vertintojams buvo nepriimtini, todėl didesnės koncentracijos tolimesniems eksperimentams buvo atmestos. Mėginiai su 1–3 % šermukšnių išspaudų, iš kurių virškriziniu CO2 pašalinti riebalai (>5 laikoma patenkinamu įvertinimu), todėl tolesniems eksperimentams buvo pasirinkta 2% koncentracija. Nepaisant tam tikro teigiamo šermukšnių išspaudų sudedamųjų dalių poveikio kokybės savybėms, jų naudojimas mėsos gaminiuose ribotas dėl poveikio juslinėms savybėms – daugiau nei 2 %

šermukšnių išspaudų ingredientų dozė buvo nepriimtina dėl neigiamo poveikio skoniui. Remiantis preliminariu jusliniu vertinimu, mėsos kukuliai su skirtingu BC sėklų ingredientų kiekiu buvo įvertinti teigiamai.

Vertiant augalinių ingredientų įtaką mikrokiekiais esančių molekulių sudėčiai buvo panaudotas vadinamasis netikslinės metabolomikos metodas. Šiuo metodu mėsos kukuliuose su BC sėklų ingredientais buvo aptikti ir preliminariai identifikuoti 749 junginiai, tarp jų keli pirminiai ir antriniai lipidų bei baltymų oksidacijos metu susidarantys žymenys – 13-L-hidroperoksilinolo rūgštis, (12*S*,13*S*)-epoksilinoleno rūgštis, 9,10-epoksioktadeceno rūgštis, glutationas, glutationo disulfidas, L-karnozinas, L-askorbo rūgštis ir kt. Taip pat buvo nustatyti fenoliniai junginiai, kurie greičiausiai patenka į mėsos gaminius su BC sėklomis. BC sėklose yra sveikos mitybos požiūriu pageidaujamų polinesočiųjų riebalų rūgščių (γ-linoleno rūgšties, α-linoleno rūgšties, linolo). Netikslinės metabolomikos metodu buvo aptikta α-linoleno rūgštis ir keli jos epoksidai bei hidroksi dariniai, greičiausiai susiję su oksidacijos procesais. Hierarchinės klasterių analizės metodu buvo aiškiai atskirtas kontrolinis mėsos kukulių gaminys nuo gaminių su skirtingomis BC sėklų ingredientų dozėmis. Statistinė analizė taip pat atskleidė, kad reikšmingiausią įtaką mėsos kukulių cheminiam profiliui turėjo 3–5 % neekstrahuotų nuriebalintų BC sėklų, iš kurių virškriziniu CO2 pašalinti riebalai, priedai. Apibendrinant galima teigti, kad tiek ingrediento dozė, tiek ir ekstrahavimas yra svarbūs veiksniai, kuriuos reikia atidžiai įvertinti, siekiant pagerinti bendrąją termiškai apdorotos kiaulienos gaminių kokybę.

Netikslingam mėsos gaminių su šermukšnių išspaudų sudedamosiomis dalimis cheminiam profiliavimui taip pat buvo panaudota hierarchinė klasterių analizė (HCA) siekiant juos sugrupuoti pagal esminius jų cheminio profilio panašumus. Pagal jų individualias gausos vertes ir sudėtinius masės spektrus (MSMS) buvo aptikti 402 junginiai, tarp jų 184 diskriminuojantys gaminius metabolitai. Mėsos metabolitų pokyčiai buvo įvertinti po 14 dienų laikymo, siekiant gauti informacijos apie galimą oksidacinių procesų poveikį mėsos komponentams ir galimą šermukšnių išspaudų sudedamųjų dalių apsauginį vaidmenį. Tam buvo sukurtas OPLS-DA modelis ir kontrolinis mėginys (C14) buvo atskirtas nuo mėginių su išspaudomis (R14, E14 ir AC14). Mėsos kukuliai su šermukšnių išspaudų sudedamosiomis dalimis turėjo tam tikrų cheminių savybių skirtumų, o gaminiai su 2 %-R (ekstrahavimo liekana) ir 1 %E (EtOH/vandens ekstraktas) buvo labai arti vienas kito. Nustatyta, kad 1 %-E (EtOH/vandens ekstraktas) priedas geriausiai apsaugojo nuo aldehidų ir ketonų kaupimosi, palyginti su kontroliniu gaminiu, po 14 laikymo dienų. Taip pat kotletuose su šermukšnių sudedamosiomis dalimis buvo nustatyti pagrindiniai fenoliniai junginiai – chlorogeno rūgštis ir izokvercitrinas. Tai patvirtina hipotezę, kad šermukšnių išspaudų priedai gali apsaugoti lipidus nuo oksidacijos procesų.

Atlikti tyrimai parodė, kad išbandyti augalinės kilmės ingredientai gali būti pripažinti perspektyviais antioksidantais, kuriuos galima naudoti mėsos gaminiuose 1–5 % koncentracijomis ir kurie gali pagerinti jų kokybės rodiklius bei mikrokiekiais esančių cheminių junginių profilį. Rezultatai taip pat rodo, kad atlikus reikiamus bandomuosius tyrimus, šiuos augalinės kilmės ingredientus galima būtų

pritaikyti pramoniniu lygmeniu. Tokį taikymą taip pat skatina tai, kad šiame darbe panaudoti augalinės kilmės ingredientai yra pigūs, prieinami ir turi maisto kokybės (GRAS) statusą. Žemės ūkio maisto perdirbimo šalutinių srautų perdirbimas į aukštos maistinės vertės ingredientus, kurie bus naudojami ir mėsos gaminiuose, tampa svarbia maisto mokslo ir technologijų tendencija. Tolesni tyrimai turėtų būti plėtojami vertinant kitas su kokybe susijusias savybes, taip pat kitų žemės ūkio ir maisto produktų gamybos šalutinių srautų perdirbimui.

REFERENCES

1. LORENZO, J.M., and M. PATEIRO. Influence of fat content on physico-chemical and oxidative stability of foal liver pâté. *Meat Science*. 2013, 95(2), 330-335. ISSN 0309-1740.
2. OECD and Food and Agriculture Organization of the United Nations. OECD-FAO Agricultural Outlook 2022-2031. [interactive]. 2022. [read: 02-06-2023]. Access via internet: https://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2022-2031_f1b0b29c-en
3. ROCCHETTI, G. et al. Meat extenders from different sources as protein-rich alternatives to improve the technological properties and functional quality of meat products. *Current Opinion in Food Science*. 2023, 49, 100967. ISSN 26659271.
4. CALDEIRA, C. *et al.* Technical Report—Food Waste Accounting Methodologies, Challenges and Opportunities; Publications Office of the European Union: Luxembourg, 2017.
5. GALANAKIS, C. (Ed.) *Valorization of Fruit Processing By-products*, 1st Edition. Academic Press ISBN: 9780128171066, London. 2019.
6. ROCCHETTI, G. et al. Impact of a pitanga leaf extract to prevent lipid oxidation processes during shelf life of packaged pork burgers: An untargeted metabolomic approach. *Foods*. 2020, 9(11), 1668. ISSN 2304-8158.
7. KOTECKA-MAJCHRZAK, K. et al. The effect of hemp cake (*Cannabis sativa* L.) on the characteristics of meatballs stored in refrigerated conditions. *Molecules*. 2021, 26(17):5284. ISSN 14203049.
8. GABDUKAEVA, L.Z. et al. Current trends in the development of functional meat products to improve the nutritional status of the population. In *IOP Conference Series: Earth and Environmental Science*. 2021, p. 012196. IOP Publishing.
9. SHAN, L.C. et al. Consumer evaluations of processed meat products reformulated to be healthier–A conjoint analysis study. *Meat Science*. 2017, 131, 82-89. ISSN 0309-1740.
10. PEREZ-MONTES, A. et al. Edible mushrooms as a novel trend in the development of healthier meat products. *Current Opinion in Food Science*. 2021, *37*, 118-124. ISSN 26659271.
11. VERBEKE, W. et al. European citizen and consumer attitudes and preferences regarding beef and pork. Meat Science. 2010, 84(2), 284-292. ISSN 0309-1740.
12. SHA, L., and Y.L. XIONG. Plant protein-based alternatives of reconstructed meat: Science, technology, and challenges. *Trends in Food Science & Technology*. 2020, 102, 51–61. ISSN 0924-2244.
13. DANOWSKA-OZIEWICZ, M. and L. KURP. Physicochemical properties, lipid oxidation and sensory attributes of pork patties with lupin protein concentrate stored in vacuum, modified atmosphere and frozen state. *Meat Science*. 2017, 131, 158–165. ISSN 0309-1740.
14. ZHANG, T. et al. The development history and recent updates on soy protein-based meat alternatives. *Trends in Food Science & Technology*. 2021a, 109, 702–710. ISSN 0924-2244.

15. VAITHIYANATHAN, S. et al. Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4 °C). *Meat Science*. 2011, 88(3), 409-414. ISSN 0309-1740.
16. JONGBERG, S. et al. Effect of white grape extract and modified atmosphere packaging on lipid and protein oxidation in chill stored beef patties. *Food Chemistry*. 2011, 128(2):276-83. ISSN 0308-8146.
17. GANHÃO, R. et al. Characterization of Selected Wild Mediterranean Fruits and Comparative Efficacy as Inhibitors of Oxidative Reactions in Emulsified Raw Pork Burger Patties. *Journal of Agricultural Food Chemistry*. 2010, 58, 15, 8854–8861. ISSN 0021-8561.
18. TAMKUTĖ, L. et al. Cranberry and black chokeberry extracts isolated with pressurized ethanol from defatted by supercritical CO_2 pomace inhibit colorectal carcinoma cells and increase global antioxidant response of meat products during *in vitro* digestion. *Food Research International*. 2022, 161, 111803. ISSN 0963-9969.
19. SARV, V. et al Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs. *Food Chemistry: X*. 2023, 19, 100761. ISSN 2590-1575.
20. NOWAK, A. et al. Polyphenolic extracts of cherry (*Prunus cerasus* L.) and blackcurrant (*Ribes nigrum* L.) leaves as natural preservatives in meat products. *Food Microbiology*. 2016, 59:142-9. ISSN 2304-8158.
21. OWUSU-ANSAH, P. et al. Non-meat ingredients in meat products: A scoping review. *Applied Food Research*. 2022, 2(1), 100044. ISSN 2772-5022.
22. ZAJAC, M. et al. The quality of pork loaves with the addition of hemp seeds, de-hulled hemp seeds, hemp protein and hemp flour. *LWT – Food Science and Technology*. 2019, 105, 190–199. ISSN 0023-6438.
23. KAUSAR, T. et al. A review on functional ingredients in red meat products. *Bioinformation*. 2019, 15(5), 358. ISSN 0973-8894.
24. PIHLANTO, A. et al. Bioactivities of alternative protein sources and their potential health benefits. *Food & Function*. 2017, 8(10):3443-3458. ISSN 2042-650X.
25. IARC. Monographs Evaluate Consumption of Red and Processed Meat. International Agency for Research on Cancer. World Health Organization [interactive]. 2015. [read: 12-06-2023]. Access via internet: https://www.iarc.who.int/wp-content/uploads/2018/07/pr240_E.pdf
26. LONGATO, E. et al. The effect of natural ingredients (amaranth and pumpkin seeds) on the quality properties of chicken burgers. *Food and Bioprocess Technology*. 2017, 10(11), 2060– 2068. ISSN 19355149.
27. DOMINGUEZ, R. et al. Effect of the partial replacement of pork backfat by microencapsulated fish oil or mixed fish and olive oil on the quality of frankfurter type sausage. *Journal of Food Science and Technology*. 2017a, 54, 26– 37. ISSN 0022-1155.
28. DOMINGUEZ, R. et al. Influence of partial pork backfat replacement by fish oil on nutritional and technological properties of liver pate. *European Journal of Lipid Science and Technology*. 2017b, 119, 1600178. ISSN 1438-9312.
29. SCHMIELE, M. et al. Dietary fiber as fat substitute in emulsified and cooked meat model system. *LWT – Food Science and Technology*. 2015, 61(1), 105-111. ISSN 0023-6438.
30. ZININA, O. et al. Enrichment of meat products with dietary fibers: a review. *Agronomy Research*. 2019, 17(4):387-396. ISSN 1406894X.

31. ANDERSON, J.W. et al. Health benefits of dietary fiber. *Nutrition Reviews*. 2009, 67(4), n188-205. ISSN 1753-4887.
32. GUSTAFSON, C.R., and D.J. ROSE. US consumer identification of the health benefits of dietary fiber and consideration of fiber when making food choices. *Nutrients*. 2022, 14(11), 2341. ISSN 20726643.
33. CUNHA, L.C. et al. Natural antioxidants in processing and storage stability of sheep and goat meat products. *Food Research International*. 2018, 111, 379-390. ISSN 09639969.
34. LORENZO, J.M. et al. Berries extracts as natural antioxidants in meat products: A review. *Food Research International*. 2018, 106, 1095-1104. ISSN 0963-9969.
35. LUNN, J., and J.L. BUTTRISS. Carbohydrates and dietary fibre. *Nutrition Bulletin*. 2007, 32(1), 21-64. ISSN 14673010, 14719827.
36. NIKMARAM, N. et al. Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. *Meat Science*. 2018, 145:245–255. ISSN 0309-1740.
37. SHEN, P. et al. Ferreting out the secrets of industrial hemp protein as emerging functional food ingredients. *Trends in Food Science & Technology*. 2021, 112, 1-15. ISSN 0924-2244.
38. KRISTENSEN, H.T. et al. Improved food functional properties of pea protein isolate in blends and co-precipitates with whey protein isolate. *Food Hydrocolloids*. 2021, 113, 106556. ISSN 0268-005X.
39. BISWAS, O. et al. Effect of phytochemicals on quality and safety aspects of meat and meat products. *Indian Journal of Animal Health*. 2021, 60, 97-108. ISSN 0019-5057.
40. MADANE, P. et al. Drumstick (*Moringa oleifera*) flower as an antioxidant dietary fibre in chicken meat nuggets. *Foods*. 2019, 8(8), 307. ISSN 2304-8158.
41. ALY, Ahmed Abdelfattah. Utilization of pomegranate peels to increase the shelf life of chicken burger during cold storage. *Egyptian Journal of Food Science*. 2019, 47(1), 1-10. ISSN 2636-4034.
42. HAM, Y.K. et al. Lotus (*Nelumbo nucifera*) rhizome as an antioxidant dietary fiber in cooked sausage: effects on physicochemical and sensory characteristics. *Korean Journal for Food Science of Animal Resources*. 2017, 37(2), p.219. ISSN 2636-0772.
43. FANG, Z. et al. Effects of incorporation of sugarcane fibre on the physicochemical and sensory properties of chicken sausage. *International Journal of Food Science & Technology*. 2018, 54, 1036– 1044. ISSN 0950-5423.
44. KIM, H.S., and K.B. CHIN. Evaluation of antioxidative activity of various levels of ethanol extracted tomato powder and application to pork patties. *Korean Journal for Food Science of Animal Resources*. 2017, 37(2), 242.
45. BASANTA, M.F. et al. Plum (*Prunus salicina*) peel and pulp microparticles as natural antioxidant additives in breast chicken patties. *Food Research International*. 2018, 106, 1086-1094. ISSN 0963-9969.
46. THOMAS, R. et al. Antioxidant and antimicrobial effects of kordoi (*Averrhoa carambola*) fruit juice and bamboo (*Bambusa polymorpha*) shoot extract in pork nuggets. *Food Chemistry*. 2016, 190, 41-49. ISSN 0308-8146.
47. STATISTICS ESTONIA. PM0281: Agricultural land and crops by county. [interactive]. 2022. [read: 02-06-2023]. Access via database: https://andmed.stat.ee/en/stat/majandus__pellumajandus__pellumajandussaaduste-tootmine__taimekasvatussaaduste-tootmine/PM0281

48. INTERNATIONAL BLACKCURRANT ASSOCIATION [interactive]. 2021. [read:04-07-2023]. Access via internet: https://www.blackcurrant-iba.com/wp-content/uploads/2021/12/Global-BC-Production-2020-21.pdf.
49. TABART, T. et al. Antioxidant and anti-inflammatory activities of *Ribes nigrum* extracts. *Food Chemistry*. 2012, 131, 1116-1122. ISSN 0308-8146.
50. XU, Y. et al. Purification, characterization and antiglycation activity of a novel polysaccharide from black currant. *Food Chemistry*. 2016, 199:694-701. ISSN 0308-8146.
51. XU, Y. et al. Characterization, antioxidant and hypoglycemic activities of degraded polysaccharides from blackcurrant (*Ribes nigrum* L.) fruits. *Food Chemistry*. 2018, 243:26-35. ISSN 0308-8146.
52. BARRECA, D. et al. Food flavonols: Nutraceuticals with complex health benefits and functionalities. *Trends in Food Science & Technology*. 2021, 117, 194-204. ISSN 0924-2244.
53. de ARAÚJO, F.F. et al. Polyphenols and their applications: An approach in food chemistry and innovation potential. *Food Chemistry*. 2021, 338, p.127535. ISSN 0308-8146.
54. KIKAS, A., and A.V. LIBEK. Maalehe sõstra-ja karusmarjaraamat. Tallinn: Hea Lugu, 2014.
55. NUTRIDATA. *Food composition database, version 12.0.* [interactive]. 2023. [read: 03-06-2023]. Access via internet: https://tka.nutridata.ee/en/
56. TIAN, Y. et al. Compositional diversity among blackcurrant (*Ribes nigrum*) cultivars originating from European countries. *Journal of Agricultural and Food Chemistry*. 2019, 67(19), 5621-5633. ISSN 0308-8146.
57. KALDMÄE, H. et al. Genotype and microclimate conditions influence ripening pattern and quality of blackcurrant (*Ribes nigrum* L.) fruit. *Zemdirbyste*. 2013, 100(2), 167–174. ISSN 13923196.
58. KIKAS, A. et al. Comparison of polyphenols and anthocyanin content of different blackcurrant (*Ribes nigrum* L.) cultivars at the Polli Horticultural Research Centre in Estonia. *Agronomy Research*. 2020, 18(4), 2715–2726. ISSN 1406-894X.
59. PETROVA, K. et al. Influence of blackcurrant variety on the quality characteristic of fruit drinks. In *International Scientific-Practical Conference „Food, Technologes and Health"*. Agricultural Academy, Food Recearch and Development Institute, 2020. pp. 95–100.
60. BISHAYEE, A. et al. Anthocyanin-rich black currant (*Ribes nigrum* L.) extract affords chemoprevention against diethylnitrosamine-induced hepatocellular carcinogenesis in rats. *The Journal of Nutritional Biochemistry*. 2011, 22(11), 1035-1046.
61. MÄÄTTÄ, K. et al. Phenolic compounds in berries of black, red, green, and white currants (Ribes sp.). *Antioxidants & Redox Signaling*. 2001, 3(6), 981–993. ISSN 1523-0864.
62. JIA, N. et al. Antioxidant activity of black currant (*Ribes nigrum* L.) extract and its inhibitory effect on lipid and protein oxidation of pork patties during chilled storage. *Meat Science*. 2012, 91(4), 533-539. ISSN 0309-1740.
63. BORGES, G. et al. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *Journal of Agricultural and Food Chemistry*. 2010, 58(7), 3901-3909. ISSN 0308-8146.

64. dos SANTOS LIMA, A. et al. From waste to the gut: Can blackcurrant press cake be a new functional ingredient? Insights on *in vivo* microbiota modulation, oxidative stress, and inflammation. *Food Research International*. 2023, 170, 112917. ISSN 09639969.
65. WU, Q.K. et al. Berry phenolic extracts modulate the expression of p21WAF1 and Bax but not Bcl-2 in HT-29 colon cancer cells. *Journal of Agricultural and Food Chemistry*. 2007, 55(4), 1156-1163. ISSN 0021-8561.
66. GOPALAN, A. et al. The health benefits of blackcurrants. *Food & Function*. 2012, 3(8), 795–809. ISSN 20426496, 2042650X.
67. JAKOBEK, L. et al. Flavonols, Phenolic Acids and Antioxidant Activity of Some Red Fruits. *Deutsche Lebensmittel-Rundschau*. 2007, 103, 369-378. ISSN 00120413.
68. VAGIRI, M.R. Phenolic Compounds and Ascorbic Acid in Black Currant (*Ribes nigrum* L.) – Variation due to genotype, ontogenetic stage, harvest date and location. Doctoral thesis, 2014. Swedish University of Agricultural Sciences, Faculty of Landscape Architecture, Horticultural and Crop Production Science, Department of Plant Breeding. Acta Universitas Agriculturae Sueciae, Alnarp. ISBN: 978-91-576-8135-5.
69. PISOSCHI, A.M. et al. An overview of natural antimicrobials role in food. *European Journal of Medicinal Chemistry*. 2018, 143, 922–935. ISSN 0223-5234.
70. PAREDES-LÓPEZ, O. et al. Berries: improving human health and healthy aging, and promoting quality life—A review. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)*. 2010, 65, 299-308. ISSN 1573-9104.
71. ANTON, D. et al. Antimicrobial and antioxidative effects of plant powders in raw and cooked minced pork. *Foods*. 2019, 8(12), 1–18. ISSN 2304-8158.
72. RAUDSEPP, P. et al. Antibacterial and antioxidative properties of different parts of garden rhubarb, black currant, chokeberry and blue honeysuckle. *Journal of the Science of Food and Agriculture*. 2018, 99, 2311–2320. ISSN 10970010, 00225142.
73. WÓJCIAK, K.M. et al. Reduction of Nitrite in Canned Pork through the Application of Black Currant (*Ribes nigrum* L.) Leaves Extract. *Molecules*. 2023, 28(4), 1749. ISSN 14203049.
74. UEYAMA, Y. et al. Volatile constituents of ethanol extracts of *Hierochloe odorata* L. var. *Pubescens* kryl. *Flavour and Fragrance Journal*. 1991, 6(1), 63-68. ISSN 0882-5734.
75. EFSA (European Food Safety Authority). Coumarin in flavourings and other food ingredients with flavouring properties. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC). EFSA J. 2008, 793, 1–15. ISSN 18314732.
76. EFSA (European Food Safety Authority). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contacts with Food (AFC) on a request from the Commission related to coumarin. EFSA J. 2004, 104, 1–36.
77. BILGIN, H.M. et al. Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. *Experimental and Toxicologic Pathology*. 2011, 63, 325–330. ISSN 0940-2993.
78. HOULT, J.R.S., and M. PAYA. Pharmacological and biochemical actions of simple coumarins: Natural products with therapeutical potential. *General Pharmacology*. 1996, 27, 713722. ISSN 1879-0011.

79. BANDONIENĖ, D. et al. Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. *Food Research International*. 2000, 33, 785–791. ISSN 0963-9969.
80. PUKALSKAS, A. et al. Identification of radical scavengers in sweet grass (*Hierochloe odorata*). *Journal of Agricultural and Food Chemistry*. 2002, 50(10), 2914-2919. ISSN 0021-8561.
81. SLAPŠYTĖ, G. et al. Evaluation of the biological activity of naturally occurring 5, 8-dihydroxycoumarin. *Molecules*. 2013, 18(4), 4419-4436. ISSN 1420-3049.
82. GRIGONIS, D. et al. Comparison of different extraction techniques for isolation of antioxidants from sweet grass (*Hierochloe odorata*). *The Journal of Supercritical Fluids*. 2005, 33(3), 223-33. ISSN 0896-8446.
83. ZAINUDDIN, A. et al. Antioxidant activity of sweetgrass (*Hierochloë odorata* Wahlnb.) extract in lard and rapeseed oil emulsions. *Food Nahrung*. 2002, 46, 15–17. ISSN 0027-769X.
84. CRINI, G. et al. Applications of hemp in textiles, paper industry, insulation and building materials, horticulture, animal nutrition, food and beverages, nutraceuticals, cosmetics and hygiene, medicine, agrochemistry, energy production and environment: A review. *Environmental Chemistry Letters*. 2020, 18, 1451–1476. ISSN 16103653.
85. ZAHARI, I. et al. Development of High-Moisture Meat Analogues with Hemp and Soy Protein Using Extrusion Cooking. *Foods*. 2020, 9, 772. ISSN 2304-8158.
86. WANG, X-S. et al. Characterisation, amino acid composition and *in vitro* Digestibility of hemp (*Cannabis sativa* L.) proteins. *Food Chemistry*. 2008, 107, 11-18. ISSN 0308-8146.
87. KOZIOŁ, M.J. Chemical composition and nutritional evaluation of quinoa (*Chenopodium quinoa* Willd.). *Journal of Food Composition and Analysis*, 5(1), 35-68.
88. MONTERO, L. et al. Hemp seeds: Nutritional value, associated bioactivities and the potential food applications in the Colombian context. *Frontiers in Nutrition*. 2023, 9, p.1039180. ISSN 2296861X.
89. TEH, S.S., and J. BIRCH. Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *Journal of Food Composition and Analysis*. 2013, 30(1), 26-31. ISSN 0889-1575.
90. XU, Y. et al. Hempseed as a nutritious and healthy human food or animal feed source: A review. *International Journal of Food Science & Technology*. 2021, 56, 530–543. ISSN 0950-5423.
91. FARINON, B. et al. The seed of industrial hemp (*Cannabis sativa* L.): Nutritional Quality and Potential Functionality for Human Health and Nutrition. *Nutrients*. 2020, 12, 1935. ISSN 20726643.
92. HOUSE, J.D. et al. Evaluating the quality of protein from hemp seed (*Cannabis sativa* L.) products through the use of the protein digestibility-corrected amino acid score method. *Journal of Agricultural Food Chemistry*. 2010, 58(22), 11801-11807. ISSN 0021-8561.
93. CHEN, H. et al. Emerging natural hemp seed proteins and their functions for nutraceutical applications. *Food Science and Human Wellness*. 2023, 12(4), 929-941. ISSN 2213-4530.
94. POTIN, F., and R. SAUREL. Hemp seed as a source of food proteins. Sustainable Agriculture Reviews 42: Hemp Production and Applications. 2020, 265-294.

95. LEONARD, W. et al. Hempseed in food industry: Nutritional value, health benefits, and industrial applications. *Comprehensive Reviews in Food Science and Food Safety*. 2020, 19(1), 282-308. ISSN 15414337.
96. MARTINEZ, J.R. et al. Characterization of bioactive compounds in defatted hempseed (*Cannabis sativa* L.) by UHPLC-HRMS/MS and anti-inflammatory activity in primary human monocytes. *Food and Functions*. 2020, 11, 4057–4066. ISSN 2042-650X.
97. REA, J. et al. Hydroxycinnamic acid derivatives isolated from hempseed and their effects on central nervous system enzymes. *International Journal of Food Sciences and Nutrition*. 2021, 72, 184–194. ISSN 0963-7486.
98. PELLATI, F. et al. *Cannabis sativa* L. and nonpsychoactive cannabinoids: their chemistry and role against oxidative stress, inflammation, and cancer. *BioMed Research International*. 2018. ISSN 2314-6141.
99. NAUMOVA, N. et al. Organoleptic evaluation of the quality of the enriched chopped semi-finished meat products. Bulletin of the Transilvania University of Brasov. *Forestry, Wood Industry, Agricultural Food Engineering*. 2017, Series II, 10, 125– 132. ISSN 2065-2135.
100. BOBINAITĖ, R. et al. Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents. *Journal of Industrial and Engineering Chemistry*. 2020, 85, 152-160. ISSN 1226086X.
101. SHIKOV, A.N. et al. Medicinal Plants of the Russian Pharmacopoeia; Their history and applications. *Journal of Ethnopharmacology*. 2014, 154, 481–536. ISSN 0378-8741.
102. BERNA, E., Kampuse, S. and E. Straumite (2012). The suitability of different rowanberry cultivars for production of fruit marmalade. In *Proceedings of the Annual 18th International Scientific Conference "Research for Rural Development*", *May 16–18 May, 2012, Jelgava, Latvia*. Latvia University of Agriculture (pp. 109–116).
103. MRKONJIĆ, Z. Et al. Phenolic profiling and bioactivities of fresh fruits and jam of Sorbus species. *Journal of the Serbian Chemical Society*. 2017, *82*(6), pp.651-664.
104. HALLMANN, E. et al. The content of biologically active compounds in some fruits from natural state. *Vegetable Crops Research Bulletin*. 2011, 75(1), 81–90. ISSN1898-7761.
105. SOŁTYS, A. et al. Ethnopharmacologically important but underestimated genus Sorbus: A comprehensive review. *Phytochemistry Reviews*. 2020, 19, 491–526. ISSN 15687767.
106. OLSZEWSKA, M.A. et al. Assessment of the content of phenolics and antioxidant action of inflorescences and leaves of selected species from the genus *Sorbus* sensu stricto. *Molecules*. 2010, 15(12), 8769-8783. ISSN 14203049.
107. BECERRA-HERRERA, M. et al. Extraction and determination of phenolic compounds in the berries of *Sorbus americana* Marsh and *Lonicera oblongifolia* (Goldie) Hook. *Food Analytical Methods*. 2015, 8, 2554–2559. ISSN 2304-8158.
108. KYLLI, P. et al. Rowanberry phenolics: Compositional analysis and bioactivities. *Journal of Agricultural and Food Chemistry*. 2010, 58, 11985–11992. ISSN 0021-8561.
109. POYRAZOĞLU, E.S. Changes in ascorbic acid and sugar content of rowanberries during ripening. *Journal of Food Quality*. 2004, 27(5), 366-370. ISSN 1745-4557.
110. SARV, V. et al. The *sorbus* spp.—underutilised plants for foods and nutraceuticals: Review on polyphenolic phytochemicals and antioxidant potential. *Antioxidants*. 2020, 9, 813. ISSN 2076-3921.

111. SARV, V. et al. Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (*Sorbus aucuparia* L.) Cultivated in Estonia. *Antioxidants*. 2021, 10(11), 1779. ISSN 2076-3921.

112. KÄHKÖNEN, M.P. et al. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*. 1999, 47(10), 3954-3962. ISSN 0021-8561.

113. HUKKANEN, A.T. et al. Antioxidant capacity and phenolic content of sweet rowanberries. *Journal of Agricultural Food Chemistry*. 2006, 54, 112–119. ISSN 0021-8561.

114. EC (2004). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. *Official Journal of the European Union*, L 139/55.

115. EESTI TOIDUAINETE TEHNOLOOGIA SELTS. *Abiks mahepõllumajandussaaduste väikekäitlejale. 2. osa, Liha töötlemine* [interactive]. 2011. ISBN: 978-9949-462-36-0. [read: 06-06-2023]. Access via internet: http://www.digar.ee/id/nlib-digar:121494.

116. REI, M. *Lihatehnoloogia teaduslikud alused*. Tartu: Kirjastus Halo, 2004.

117. OLAOYE, Olusegun Ayodele. Meat: An overview of its composition, biochemical changes and associated microbial agents. *International Food Research Journal*. 2011, 18(3). ISSN 22317546.

118. SALLAM, K.I. et al. Antioxidant and antimicrobial effects of garlic in chicken sausage. *LWT - Food Science and Technology*. 2004, 37(8), 849– 855. ISSN 0023-6438

119. JAKOBSEN, M., and G. BERTELSEN. The use of CO_2 in packaging of fresh red meats and its effect on chemical quality changes in the meat: a review. *Journal of Muscle Foods*. 2002, 13(2), 143–168. ISSN 10460756

120. WARRISS, P.D. *Meat science: an introductory text*. Guildford ja King's Lynn: CABI publishing, 2000. ISBN 978-1-84593-593-1.

121. MACDOUGALL, D.B. Colour of meat. Quality attributes and their measurement in meat, poultry and fish products. *Advances in Meat Series.* 1994, 79-93.

122. NARASIMHA RAO, D., and N.M. SACHINDRA. Modified Atmosphere and Vacuum Packaging of Meat and Poultry Products. *Food Reviews International*. 2002, 18(4), 263–293. ISSN 8755-9129.

123. KOSOWSKA, M. et al. Volatile compounds in meat and meat products. *Food Science and Technology*. 2017, 37, 1-7. ISSN 00221155, 09758402.

124. TAMANNA, N., and N. MAHMOOD. Food processing and Maillard reaction products: effect on human health and nutrition. *International Journal of Food Science*. 2015, ISSN 0950-5423.

125. AASLYNG, M.D. et al. Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preference*. 2003, *14*(4), 277-288. ISSN 17454557, 01469428.

126. ROSS, C.F., and D.M. SMITH. Use of volatiles as indicators of lipid oxidation in muscle foods. *Comprehensive Reviews in Food Science and Food Safety*. 2006, 5(1), 18-25. ISSN 15414337.

127. HUANG, X., and D.U. AHN. Lipid oxidation and its implications to meat quality and human health. *Food Science and Biotechnology*. 2019, 28(5), 1275–1285. ISSN 1226-7708.

128. ESTÉVEZ, Mario., David S. MORCUENDE, Sonia, VENTANAS. *Determination of Oxidation*. In Handbook of Muscle Foods Analysis, 1st ed. CRC Press: Boca Raton, FL, USA, 2009.

129. MASUDA T., Y. INABA, Y. TAKEDA. Antioxidant mechanism of carnosic acid: Structural identification of two oxidation products. *Journal of Agricultural and Food Chemistry*. 2001, 49: 5560– 5. ISSN 1520-5118.

130. ELSHAFIE, H.S., and I. CAMELE. An overview of the biological effects of some mediterranean essential oils on human health. *BioMed Research International*. 2017. ISSN 2314-6133.

131. NAVIGLIO, D. et al. Rapid Solid-Liquid Dynamic Extraction (RSLDE): A powerful and greener alternative to the latest solid-liquid extraction techniques. *Foods*. 2019, *8*(7), 245. ISSN 2304-8158.

132. KOINA, I.M. et al. Green Extraction Techniques for the Determination of Active Ingredients in Tea: Current State, Challenges, and Future Perspectives. *Separations*. 2023, 10(2), 121. ISSN 2297-8739.

133. ANASTAS, P., and N. EGHBALI. Green chemistry: principles and practice. *Chemical Society Reviews*. 2010. 39(1), 301-312. ISSN 03060012, 14604744.

134. AWAD, A.M. et al. Overview of plant extracts as natural preservatives in meat. *Journal of Food Processing and Preservation*. 2022, 46(8), p.e16796. ISSN 1745-4549.

135. PATEIRO, M. et al. Plant extracts obtained with green solvents as natural antioxidants in fresh meat products. *Antioxidants*. 2021, 10(2), 181. ISSN 20763921.

136. PICOT-ALLAIN, M.C.N. et al. Extraction, characterisation, and application of pectin from tropical and sub-tropical fruits: a review. *Food Reviews International*. 2022, 38(3), 282-312.

137. KITRYTĖ, V. et al. Zero waste biorefining of lingonberry (*Vaccinium vitis-idaea* L.) pomace into functional ingredients by consecutive high pressure and enzyme assisted extractions with green solvents. *Food Chemistry*. 2020, 322, 126767. ISSN 0308-8146.

138. TAMKUTĖ, L. Et al. Recovery of valuable lipophilic and polyphenolic fractions from cranberry pomace by consecutive supercritical CO_2 and pressurized liquid extraction. *The Journal of Supercritical Fluids*. 2020, 159, 104755. ISSN 0896-8446.

139. BIRLA, R. et al. Storage stability of pork emulsion incorporated with Arjuna (*Terminalia arjuna*) tree bark extract. *International Journal of Livestock Research*. 2019, 9(1). ISSN 2277-1964.

140. RATHOUR, M. et al. Standardization of protocols for extraction of aloe vera and cinnamon bark extracts. *Journal of Animal Research*. 2017, 7(1), 175-182. ISSN 2277-940X.

141. BEYA, M.M. et al. Plant-based phenolic molecules as natural preservatives in comminuted meats: A review. *Antioxidants*. 2021, 10(2), 263. ISSN 20763921.

142. BASEGMEZ, H.I.O. et al. Biorefining of blackcurrant pomace into high value functional ingredients using supercritical CO_2, pressurized liquid and enzyme assisted extractions. *The Journal of Supercritical Fluids*. 2017, 124, 10-19. ISSN 0896-8446.

143. IBAÑEZ, E., Miguel HERRERO, Jose A. MENDIOLA, Maria CASTRO-PUYANA. (2011). *Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates*. In Marine bioactive compounds: sources, characterization and applications (pp. 55-98). Boston, MA: Springer US.

144. KADAM, S.U. et al. Application of novel extraction technologies for bioactives from marine algae. *Journal of Agricultural and Food Chemistry*. 2013, 61(20), 4667-4675. ISSN 0021-8561.

145. HALIM, R., Michael K. DANQUAH, Paul W. WEBLEY. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnology Advances*.2012, 30(3), 709-732.

146. HOSIKIAN, A. et al. Chlorophyll extraction from microalgae: A review on the process engineering aspects. *International Journal of Chemical Engineering*. 2010, 391632. ISSN 6878078, 1687806X.

147. HAHN, T. et al. Novel procedures for the extraction of fucoidan from brown algae. *Process Biochemistry*. 2012, 47(12), 1691-1698. ISSN 1359-5113.

148. SHANG, Y.F. et al. Pressurized liquid method for fucoxanthin extraction from *Eisenia bicyclis* (Kjellman) Setchell. *Journal of Bioscience and Bioengineering*. 2011, 111(2), 237-241. ISSN 13474421, 13891723.

149. KLEJDUS, B. et al. Hyphenated technique for the extraction and determination of isoflavones in algae: Ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. *Journal of Chromatography A*. 2010, 1217(51), 7956-7965. ISSN 0021-9673.

150. BELWAL, T. et al. Optimization extraction conditions for improving phenolic content and antioxidant activity in *Berberis asiatica* fruits using response surface methodology (RSM). *Food Chemistry*. 2016, 207, 115-124. ISSN 0308-8146.

151. HARTATI, S.L. et al. Optimization of supercritical CO_2 extraction of Swietenia mahagoni seed by response surface methodology. *Jurnal Teknologi (Sciences and Engineering)*. 2014, 67(1), 15-20. ISSN 21803722.

152. WRONA, O. et al. Supercritical fluid extraction of bioactive compounds from plant materials. *Journal of AOAC International*. 2017, 100:1624–1635. ISSN 10603271.

153. KHAW, K.Y. et al. Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. *Molecules*. 2017, 22(7), 1186. ISSN 1420-3049.

154. GALLO, M. et al. Antioxidant addition to prevent lipid and protein oxidation in chicken meat mixed with supercritical extracts of Echinacea angustifolia. *The Journal of Supercritical Fluids*. 2012, 72 (Supplement C), 198-204. ISSN 0896-8446.

155. PÉRINO-ISSARTIER, S. et al. Solvent free microwave-assisted extraction of antioxidants from sea buckthorn (*Hippophae rhamnoides*) food by-products. *Food and Bioprocess Technology*. 2011, 4, 1020-1028. ISSN 19355149, 19355130.

156. MICHALAK, I., and K. CHOJNACKA. Algal extracts: Technology and advances. *Engineering in Life Sciences*. 2014, 14(6), 581-591. ISSN 16182863.

157. CINTAS, P. et al. *Pharmaceutical and nutraceutical compounds from natural matrices. In Microwave-Assisted Extraction for Bioactive Compounds*. Chemat, F., Cravotto, G., Eds. Springer: New York. 2013, 4, 181– 206. ISSN 0967-0912.

158. BAKIĆ, M.T. et al. Effect of microwave-assisted extraction on polyphenols recovery from tomato peel waste. *Acta Chimica Slovenica*. 2019, 13;66(2):367-77. ISSN 15803155.

159. SOLABERRIETA, I. et al. Valorization of aloe vera skin by-products to obtain bioactive compounds by microwave-assisted extraction: antioxidant activity and chemical composition. *Antioxidants*. 2022, 11(6), 1058. ISSN 20763921.

160. KURTULBAŞ, E. et al. Microwave-assisted extraction of bioactive components from peach waste: Describing the bioactivity degradation by polynomial regression. *Biomass Conversion and Biorefinery*. 2022, 11:1-1. ISSN 21906815.

161. HORNING, E.C., and M.G. HORNING. Metabolic profiles: Chromatographic methods for isolation and characterization of a variety of metabolites in man. *Methods Medical Research*. 1970, 12, 369–371. ISSN 0962-2802.

162. NICHOLSON, J.K. et al. Metabonomics: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica.* 1999, 29(11), 1181–1189. ISSN 00498254.

163. FIEHN, Oliver. Metabolomics—The link between genotypes and phenotypes. *Plant Molecular Biology*. 2002, 48, 155–171. ISSN 2223-7747.

164. RAMANATHAN, R. et al. The potential of metabolomics in meat science: Current applications, future trends, and challenges. *Journal of Proteomics*. 2023, 104926. ISSN 1874-3919.

165. CAPOZZI, Francesco, Alessia TRIMIGNO, Pasquale FERRANTI. *Proteomics and metabolomics in relation to meat quality. In Poultry Quality Evaluation.* Woodhead Publishing, 2017 (pp. 221-245). ISSN 01458892, 17454549.

166. AGIN, A. et al. Metabolomics–an overview. From basic principles to potential biomarkers (part 1). *Médecine Nucléaire*. 2016, 40(1), 4-10. ISSN: 1878-6820.

167. BEALE, David J., Konstantinos A. KOUREMENOS, Enzo A. PALOMBO. *Microbial metabolomics: Applications in clinical, environmental, and industrial microbiology. Microbial Metabolomics: Applications in Clinical, Environmental, and Industrial Microbiology.* Springer International Publishing, 2016.

168. BEAUCLERCQ, S. et al. Serum and muscle metabolomics for the prediction of ultimate pH, a key factor for chicken-meat quality. *Journal of Proteome Research*. 2016, 15(4), 1168-1178. ISSN 1535-3893.

169. WANG, Q. et al. Exploring the formation mechanism of off-flavor of irradiated yak meat based on metabolomics. *Food Chemistry: X*. 2022, 16, 100494. ISSN 2590-1575.

170. ZHANG, T. et al. Current state of metabolomics research in meat quality analysis and authentication. *Foods*. 2021b, 10(10), 2388. ISSN 2304-8158.

171. TAMKUTE, L. et al. Effects of chokeberry extract isolated with pressurized ethanol from defatted pomace on oxidative stability, quality and sensory characteristics of pork meat products. *LWT – Food Science and Technology*. 2021, 150. ISSN 0023-6438.

172. VENSKUTONIS, P.R. Berries. In Valorization of Fruit Processing By-products, 1st ed. Ed. C. Galanakis, Academic press: London, UK, 2020; pp. 95–125. IHRN 9780128173732.

173. SINGLETON, V.L. et al. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology.* 1999, 299, 152–178. ISSN 0076-6879.

174. KITRYTĖ, V. et al. Assessment of antioxidant capacity of brewer's spent grain and its supercritical carbon dioxide extract as sources of valuable dietary ingredients. *Journal of Food Engineering*. 2015, 167, 18–24. ISSN 0260-8774.

175. RE, R. et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 1999, 26(9–10), 1231–1237. ISSN 0891-5849.

176. KERNER, K. et al. Evaluation of different blackcurrant seed ingredients in meatballs by using conventional quality assessment and untargeted metabolomics. *Meat Science*. 2023, 200, 109160. ISSN 0309-1740.

177. KERNER, K. et al. Application of Raw and Defatted by Supercritical CO_2 Hemp Seed Press-Cake and Sweet Grass Antioxidant Extract in Pork Burger Patties. *Foods*. 2021, 10(8), 1904. ISSN 2304-8158.

178. PRIOR, R.L. et al. Assays for Hydrophilic and Lipophilic Antioxidant Capacity (oxygen radical absorbance capacity (ORAC FL)) of Plasma and Other Biological and Food Samples. *Journal of Agricultural and Food Chemistry*. 2003, 51(11), 3273–3279. ISSN 0021-8561.

179. DÁVALOS, A. et al. Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC−Fluorescein) Assay. *Journal of Agricultural and Food Chemistry*. 2004, 52, 48–54. ISSN 0021-8561.

180. BRAND-WILLIAMS, W. et al. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology. 1995*, 28, 25–30. ISSN 0023-6438.

181. MOKRZYCKI, W., and M. TATOL. Color difference ΔE - A survey. Faculty of Mathematics and Informatics. *Machine Graphics and Vision*. 2011, *20*(4), 383–411. ISSN 2720250X.

182. PIKUL, J. et al. Evaluation of three modified TBA methods for measuring lipid oxidation in nchicken meat. *Journal of Agricultural and Food Chemistry*. 1989, 37, 1309–1313. ISSN 0021-8561.

183. PAVLIDIS, D.E. et al. A volatilomics approach for off-line discrimination of minced beef and pork meat and their admixture using HS-SPME GC/MS in tandem with multivariate data analysis. *Meat Science*. 2017, 151, 43-53. ISSN 0309-1740.

184. ADAMS, R.P. (Ed.) *Identification of essential oil components by gas chromatography/mass spectrometry*, 4th ed. Allured Publishing, 2017.

185. WICHCHUKIT, S., and M. O'MAHONY. The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. *Journal of the Science of Food and Agriculture*. 2015, 95(11), 2167-2178. ISSN 1097-0010.

186. PATEIRO, M. et al. Guarana seed extracts as a useful strategy to extend the shelf life of pork patties: UHPLC-ESI/QTOF phenolic profile and impact on microbial inactivation, lipid and protein oxidation and antioxidant capacity. *Food Research International*. 2018, 114, 55–63. ISSN 0963-9969.

187. ROCCHETTI, G. et al. A combined metabolomics and peptidomics approach to discriminate anomalous rind inclusion levels in Parmigiano Reggiano PDO grated hard cheese from different ripening stages. *Food Research International*. 2021, 149, 110654. ISSN 0963-9969.

188. R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.%0A

189. LENTH, R. (2022). Emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.7.3. Access via internet: https://cran.r-project.org/package=emmeans.

190. HOTHORN, T. et al. Simultaneous inference in general parametric models. *Biometrical Journal*. 2008, 50(3), 346–363. ISSN 03233847, 15214036.

191. WICKHAM, H. ggplot2: Elegant Graphics for Data Analysis; Springer: New York, NY, USA, 2016; ISBN 9783319242774. Available online: https://ggplot2.tidyverse.org

192. ALBA, K. et al. Fractionation and characterisation of dietary fibre from blackcurrant pomace. *Food Hydrocolloids.* 2018, 81, 398-408. ISSN 0268-005X.

193. LU, Y., and L. YEAP FOO. Polyphenolic constituents of blackcurrant seed residue. *Food Chemistry*. 2003, 80(1), 71–76. ISSN 0308-8146.

194. JURČAGA, L. et al. Blackcurrant (*Ribes nigrum* L.) and Kamchatka Honeysuckle (*Lonicera caerulea* var. Kamtschatica) Extract Effects on Technological Properties, Sensory Quality, and Lipid Oxidation of Raw-Cooked Meat Product (Frankfurters). *Foods*. 2021, 10(12), 2957. ISSN 2304-8158.

195. JURIKOVA, T. et al. Polyphenolic profile of interspecific crosses of rowan (*Sorbus aucuparia* L.). *Italian Journal of Food Science*. 2014, 26, 317–324. ISSN 1120-1770.

196. KAMPUSS, K. et al. Biochemical composition and antiradical activity of rowanberry (*Sorbus* L.) cultivars and hybrids with different *Rosaceae* L. cultivars. *European Journal of Horticultural Science*. 2009, 59, 195–201. ISSN 16114426.

197. SUN, G. et al. Effects of incorporation of hempseed meal on the quality attributes of chicken sausage. *Future Foods*. 2022, 6, 100169. ISSN 2666-8335.

198. RAIKOS, V. et al. Comparative study of the functional properties of lupin, green pea, fava bean, hemp, and buckwheat flours as affected by pH. *Food Science & Nutr*ition. 2014, 2, 802–810. ISSN 2470-1076.

199. CHOI, Y.S. et al. Effects of *Laminaria japonica* on the physico-chemical and sensory characteristics of reduced-fat pork patties. *Meat Science*. 2012, 91(1), 1–7. ISSN 0309-1740.

200. MENA, B. et al. Influence of cooking method, fat content and food additives on physicochemical and nutritional properties of beef meatballs fortified with sugarcane fibre. *International Journal of Food Science & Technology*. 2020, *55*(6), 2381-2390. ISSN 09505423, 13652621.

201. FONT-I-FURNOLS, M., and L. GUERRERO. Consumer preference, behavior and perception about meat and meat products: An overview. *Meat Science*. 2014, 98, 361–371. ISSN 0309-1740.

202. LEONARD, W. et al. Effects of incorporating roasted lupin (*Lupinus angustifolius*) flour on the physicochemical and sensory attributes of beef sausage. *International Journal of Food Science & Technology*. 2019, *54*(5), 1849-1857. ISSN 09505423, 13652621.

203. CARVALHO, F.A.L. et al. Replacement of meat by spinach on physicochemical and nutritional properties of chicken burgers. *Journal of Food Processing and Preservation*. 2019, 43(5), e13935. ISSN 0309-1740.

204. SCHMIDT, M.M. et al. Development of cooked and smoked chicken sausage with reduced sodium and fat. *Journal of Applied Poultry Research*. 2017, 26(1), 130–144. ISSN 1056-6171.

205. ŠIMERDOVÁ, B. et al. Evaluation of anthocyanin profiles in various blackcurrant cultivars over a three-year period using a fast HPLC-DAD method. *Foods*. 2021, 10(8), 1745. ISSN 2304-8158.

206. CHUNG, Y.K. et al. Physicochemical and storage characteristics of Hanwoo Tteokgalbi treated with onion skin powder and blackcurrant powder. *Korean Journal for Food Science of Animal Resources*. 2018, 38(4), p.737. ISSN 2636-0780.

207. DE SANTOS, F. et al. Effect of carbon monoxide in modified atmosphere packaging, storage time and endpoint cooking temperature on the internal color of enhanced pork. *Meat Science*. 2007, 77(4), 520-528.

208. ESMER, O.K. et al. The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Science*. 2011, 88(2), 221-226. ISSN 0309-1740.

209. PEIRETTI, P.G. et al. The effect of blueberry pomace on the oxidative stability and cooking properties of pork patties during chilled storage. *Journal of Food Processing and Preservation*. 2020, 44(7), p.e14520. ISSN 01458892.

210. SELANI, M.M. et al. Wine industry residues extracts as natural antioxidants in raw and cooked chicken meat during frozen storage. *Meat Science*. 2011, 88, 397– 403. ISSN 0309-1740.

211. CIE. International commission on illumination, recommendations on uniform colour spaces, colour difference equations, psychometric colour terms. CIE Publication No. 15 (E-1.3. 1) 1971/(TO-1.3), 1978. ISSN 1099-839X.

212. BARCENILLA, C. et al. Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. *Meat Science*. 2022, 183. ISSN 0309-1740.

213. HAUTRIVE, T.P. et al. Effect of fat replacement by chitosan and golden flaxseed flour (wholemeal and defatted) on the quality of hamburgers. *LWT – Food Science and Technology*. 2019, 102, 403–410. ISSN 0023-6438.

214. SÁNCHEZ-ZAPATA, E. et al. Effect of tiger nut fibre on quality characteristics of pork burger. *Meat Science*. 2020, 85(1), 70–76. ISSN 0309-1740.

215. BARRÓN-AYALA, C.G. et al. Pork frankfurters prepared with hydrolyzed whey: Preliminary product quality aspects and inhibitory activity of the resulting peptides on angiotensin-converting enzyme. *Meat Science*. 2020, 166, 108111. ISSN 0309-1740.

216. KUMAR, Y., and V. KUMAR. Effects of double emulsion (W1/O/W2) containing encapsulated *Murraya koenigii* berries extract on quality characteristics of reduced-fat meat batter with high oxidative stability. *LWT – Food Science and Technology*. 2020, 127, 109365. ISSN 0023-6438.

217. HECK, R.T. et al. Is it possible to produce a low-fat burger with a healthy n− 6/n− 3 PUFA ratio without affecting the technological and sensory properties? *Meat Science*. 2017, 130, 16-25. ISSN 0309-1740.

218. TAMKUTĖ, L. et al. Effect of cranberry pomace extracts isolated by pressurized ethanol and water on the inhibition of food pathogenic/spoilage bacteria and the quality of pork products. *Food Research International*. 2019, 120, 38-51. ISSN 0963-9969.

219. HALAGARDA, M., and K.M. WÓJCIAK. Health and safety aspects of traditional European meat products. A review. *Meat Science*. 2022, 184. ISSN 0309-1740.

220. JABERI, R. et al. The effect of barberry (Berberis vulgaris L.) extract on the physicochemical properties, sensory characteristics, and volatile compounds of chicken frankfurters. *Journal of Food Processing and Preservation*. 2020, 44(7), p.e14501. ISSN 1745-4549.

221. MÄKILÄ, L. et al. Stability of hydroxycinnamic acid derivatives, flavonol glycosides, and anthocyanins in black currant juice. J*ournal of Agricultural and Food Chemistry*. 2016, 64(22), 4584– 4598. ISSN 15205118, 00218561.

222. FOURATI, M. et al. Variability in phytochemical contents and biological potential of pomegranate (*Punica granatum*) peel extracts: toward a new opportunity for minced beef meat preservation. *Journal of Food Quality*. 2020, 1-14. ISSN 1745-4557.

223. WOOD, J.D. et al. Effects of fatty acids on meat quality: a review. *Meat Science*. 2004, 66(1), 21-32. ISSN 0309-1740.

224. TUORILA, H., and E. MONTELEONE. Sensory food science in the changing society: Opportunities, needs, and challenges. *Trends in Food Science & Technology*. 2009, 20, 54-62. ISSN 0924-2244.

225. BORTOLOTTO, F.C.K. et al. Combined effects of garlic essential oil and allyl isothiocyanate against *Escherichia coli* O157: H7 *in vitro* and in pork sausage. *Ciência Rural*. 2018, 48. ISSN 0103-8478.

226. GONZALEZ, G.S. et al. Sensory evaluation of a highly nutritive bread, formulated for populations suffering food emergencies, preserved with ionizing radiation. In *XXXVIII Annual meeting of the Argentine Association of Nuclear Technology*, 2011.

227. MICHALCZYK, M. et al. Effect of adding essential oils of caraway and rosemary on volatile aroma compounds derived from stored vacuum packaged minced Turkey meat. *Annals of Animal Science*. 2020, 21(3), 1133-1147. ISSN 23008733.

228. SAM, F.E. et al. Physicochemical, oxidative stability and sensory properties of frankfurter-type sausage as influenced by the addition of carrot (*Daucus carota*) paste. *Foods*. 2021, 10(12), 3032. ISSN 2304-8158.

229. SONG, X.C. et al. Comparison of two antioxidant packaging based on rosemary oleoresin and green tea extract coated on polyethylene terephthalate for extending the shelf life of minced pork meat. *Food Packaging and Shelf Life*. 2020, 26, p.100588. ISSN 22142894.

230. STOBNICKA, A., and M. GNIEWOSZ. Antimicrobial protection of minced pork meat with the use of Swamp Cranberry (*Vaccinium oxycoccos* L.) fruit and pomace extracts. *Journal of Food Science and Technology*. 2018, 55, 62-71. ISSN 0022-1155.

LIST OF PUBLICATIONS

1. **Kerner, K.**, Jõudu, I., Tänavots, A., Venskutonis, P. R. 2021. Application of raw and defatted by supercritical CO_2 hemp seed press-cake and sweet grass antioxidant extract in pork burger patties. Foods, 10 (8), 1904. DOI: 10.3390/foods10081904.
2. **Kerner, K.,** Kazernavičiūtė, R., Jõudu, I., Rocchetti, G., Lucini, L., Tänavots, A., Hussain, S., Venskutonis, P. R. 2023. Evaluation of different blackcurrant seed ingredients in meatballs by using conventional quality assessment and untargeted metabolomics. Meat Science, 200, 109160. DOI: 10.1016/j.meatsci.2023.109160.
3. Sarv, V., **Kerner, K.,** Venskutonis, P. R., Rocchetti, G., Becchi, P. P., Lucini, L., Tänavots, A., Bhat, R. 2023. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs. Food Chemistry: X, 100761. DOI: 10.1016/j.fochx.2023.100761.

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2. **Kerner, Kristi**; Kazernavičiūtė, Rita; Jõudu, Ivi; Rocchetti, Gabriele; Lucini, Luigi; Tänavots, Alo; Hussain, Shehzad and Venskutonis, Petras Rimantas. Evaluation of different blackcurrant seed ingredients in meatballs by using conventional quality assessment and untargeted

metabolomics // Meat Science. 2023, Vol. 200, 109160. ISSN: 0309-1740. (IF 7.077 (2023)).

3. Sarv, Viive; **Kerner, Kristi;** Venskutonis, Petras Rimantas; Rocchetti, Gabriele; Becchi, Pier Paolo; Lucini, Luigi; Tänavots, Alo and Bhat, Rajeev. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs // Food Chemistry: X. 2023, Vol. 19, 100761. ISSN: 2590-1575. (IF 6.443 (2023)).

Other publications not related to the topic of dissertation:

1. Rocchetti, Gabriele; Ferronato, Giulia; Sarv, Viive; **Kerner, Kristi**; Venskutonis, Petras Rimantas; Lucini, Luigi. Meat extenders from different sources as protein-rich alternatives to improve the technological properties and functional quality of meat products // Current Opinion in Food Science. 2023, 49, 100967. ISSN: 26659271. (IF 9.8 (2022–2023)).
2. Roasto, Mati; Mäesaar, Mihkel; Püssa, Tõnu; Anton, Dea; Rätsep, Reelika; Elias, Terje; Jortikka, Salli; Pärna, Merilin; Kapp, Karmen; Tepper, Marek; **Kerner, Kristi**; Meremäe, Kadrin. The Effect of Fruit and Berry Pomaces on the Growth Dynamics of Microorganisms and Sensory Properties of Marinated Rainbow Trout // Microorganisms. 2023, 11(12), 2960. ISSN: 2076-2607. (IF 4.5 (2022)).

Scientific conferences:

1. **Kerner, Kristi**; Venskutonis, Petras Rimantas; Jõudu, Ivi; Tänavots, Alo. Effect of sweet grass extract on oxidative stability of burger patties with mechanically pressed and defatted hemp seed additives // 14th Baltic Conference on Food Science and Technology "Sustainable Food for Conscious Consumer" (FoodBalt 2021), May 3–5, 2021, Tallinn, Estonia. Abstract book/Center of Food and Fermentation Technologies. Tallinn: Center of Food and Fermentation Technologies, 2021, p. 122.
2. **Kerner, Kristi**; Jõudu, Ivi; Tänavots, Alo; Venskutonis, Petras Rimantas. Application of raw and defatted hempseed press-cake and sweetgrass antioxidant extract in pork burger patties // XXI EuroFoodChem Conference, November 22–24, 2021, online conference.
3. **Kerner, Kristi**; Ambos, Kati; Kaart, Tanel; Venskutonis, Petras Rimantas; Jõudu, Ivi. The effect of hemp protein concentrate on the physico-chemical and sensory properties of meatballs // 15th Baltic Food Science and Technology Conference "FOODBALT-2022" "Food Research and Development in the Baltic States and Beyond", September 26–27, 2022, Kaunas, Lithuania. Abstract book KTU Department of Food Science and Technology. Kaunas: Department of Food Science and Technology, 2022, p. 60.

4. Sarv, Viive; **Kerner, Kristi**; Venskutonis, Petras Rimantas; Rätsep, Reelika; Rocchetti, Gabriele; Becchi, Pier Paolo; Lucini, Luigi; Tänavots, Alo; Bhat, Rajeev. Rowan fruit pomace as functional ingredient in meatballs formulation // 14th International Conference and Exhibition on Nutraceuticals and Functional Foods, October 2–5, 2022, İstanbul, Türkiye. Book of Abstracts 2022 Annual Conference & Exhibition Functional Foods, Nutraceuticals, Natural Health Products, and Dietary Supplements, p. 81.
5. **Kerner, Kristi**; Kazernavičiūtė, Jõudu, Ivi; Tänavots, Alo; Venskutonis, Petras Rimantas. Effect of different blackcurrant seed ingredients on the quality of pork meatballs // 16th Baltic Conference on Food Science and Technology Foodbalt 2023 "Traditional meets non-traditional in future food" (Foodbalt 2023), May 11–12, 2023, Jelgava, Latvia. Abstract book LBTU, Faculty of Food Technology, 2023, p. 107.
6. Sarv, Viive; **Kerner, Kristi**; Venskutonis, Petras Rimantas; Rocchetti, Gabriele; Becchi, Pier Paolo, Lucini, Luigi; Tänavots, Alo; Bhat, Rajeev. Evaluation of rowan fruit pomace ingredients in meatballs by conventional quality characterization and UHPLC-QTOF-MS based untargeted metabolomics with multivariate data analysis // 16th Baltic Conference on Food Science and Technology Foodbalt 2023 "Traditional meets non-traditional in future food" (Foodbalt 2023), May 11–12, 2023, Jelgava, Latvia. Abstract book LBTU, Faculty of Food Technology, 2023, p. 106.
7. **Kerner, Kristi**; Sarv, Viive; Jõudu, Ivi; Tänavots, Alo; Venskutonis, Petras Rimantas. Effect of blackcurrant skin ingredients on the physicochemical properties of pork meatballs // XXII EuroFoodChem Conference, June 14-16, 2023, Belgrade, Serbia. Book of Abstracts, Serbian Chemical Society, EuChemS, Division of Food Chemistry, 2023, p. 167.
8. **Kerner, Kristi**; Kazernavičiūtė, Rita; Jõudu, Ivi; Rocchetti, Gabriele; Lucini, Luigi; Tänavots, Alo; Hussain, Shehzad; Venskutonis, Petras Rimantas. Effect of different blackcurrant seed ingredients in meatballs by using conventional quality assessment and untargeted metabolomics // 3rd Food Chemistry Conference, October 10–12, 2023, Dresden, Germany.

Other presentations:

1. **Kerner, Kristi.** Development and Application of Vegetable Origin Additives for Improving Safety and Health Benefits of Meat Products // Baltic University Programme 7th PhD Students Training Interdisciplinary–Multicultural–International, 24–28 November, 2019, Rogów, Poland.
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3. **Kerner, Kristi**; Sarv, Viive; Tänavots, Alo, Venskutonis, Petras Rimantas. Pihlakamarjade pressjäägi kasutamine sealihast lihapallides (*Application of*

rowan berry pomace in pork meatballs) // Terve loom ja tervislik toit 2023, March 1–2, 2023, Tartu, Estonia. Konverentsi Terve Loom ja Tervislik Toit 2023 artiklite kogumik (Book of articles): Terve Loom ja Tervislik Toit 2023. Eesti Maaülikool, 2023, p. 25–33.

4. Meremäe, Kadrin; Roasto, Mati; Anton, Dea; Tepper, Marek; Elias, Terje; **Kerner, Kristi**; Rätsep, Reelika; Püssa, Tõnu. Taimsete lisandite mõju verivorstide säilivusele ja kvaliteedile (*Effect of plant additives on the shelf life and quality of blood sausages*). Terve loom ja tervislik toit 2023, March 1–2, 2023, Tartu, Estonia. Konverentsi Terve Loom ja Tervislik Toit 2023 artiklite kogumik (Book of articles): Terve Loom ja Tervislik Toit 2023. Eesti Maaülikool, p. 66−75.
5. Rätsep, Reelika; Roasto, Mati; Anton, Dea; Meremäe, Kadrin; Tepper, Marek; Kaldmäe, Hedi; Bleive, Uko; **Kerner, Kristi**; Sünter, Alar; Aluvee, Alar; Rusalepp, Linda; Koskar, Julia; Sarv, Viive; Venskutonis, Petras Rimantas; Püssa, Tõnu (2023). Ülevaade RESTA14 projekti TAIMLOOMTOIT tulemustest – liha- ja kalatoodetele lisandväärtuse andmine aiandustootmises tekkivate mahlapressjääkidega (*RESTA14 project TAIMLOOMTOIT – the valorisation of plant-based powders for value-added meat and fish products*). Terve loom ja tervislik toit 2023, March 1–2, 2023, Tartu, Estonia. Konverentsi Terve Loom ja Tervislik Toit 2023 artiklite kogumik (Book of articles): Terve Loom ja Tervislik Toit 2023. Eesti Maaülikool, p. 18−24.

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