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Doctoral Thesis

Riga, 2023

Linards Kļaviņš

BIOREFINING OF *VACCINIUM* BERRIES AND THEIR PRESS RESIDUES TO OBTAIN BIOACTIVE FUNCTIONAL INGREDIENTS

Promocijas darba
kopsavilkums

VACCINIUM ĢINTS OGU UN TO SPIEDPALIEKU
BIORAFINĒŠANA BIOLOĢISKI AKTĪVU,
FUNKCIONĀLU SASTĀVDAĻU IEGŪŠANAI

UNIVERSITY
OF LATVIA

FACULTY OF GEOGRAPHY AND EARTH SCIENCES
DEPARTMENT OF ENVIRONMENTAL SCIENCE

LINARDS KĻAVIŅŠ

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Doctoral Thesis

Submitted for the degree of Doctor of Science (Ph.D.)
in Natural Sciences
(in the field of Earth Sciences, Physical Geography
and Environmental Sciences)

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Maybe that’s enlightenment enough: to know that there is no final resting place of the mind, no moment of smug clarity. Perhaps wisdom … is realizing how small I am, and unwise, and how far I have yet to go.

Anthony Bourdain

Abstract

Berry juice processing produces large amounts of food waste – berry press residues, which consists of berry skins and seeds, potentially containing valuable components and ingredients that could be used in other industries. Berry press residues could be valorised through approaches defined by biorefinery concepts, adding additional value and obtaining new, application-based extracts or their fractions. The aim of this thesis was to evaluate the possibility to extract lipids and polyphenolics from berry press residues and explore the application potential of these groups of substances. Lipid, including, berry cuticular wax, compositional analysis revealed presence of large number of lipophilic substances with various functions. The application of lipid extracts was explored – specific fractions of lipid and wax extracts could be used as antimicrobial agents in cosmetics or as additives in sunscreens to increase the UV-B blocking potential. Polyphenolic extraction was optimised using Response Surface Methodology to achieve maximum extraction yields, concentrating on anthocyanins, which is the largest polyphenolic group found in the berries. Polyphenolic extracts have been analytically characterised showing the potential for berry press residue phytochemical extraction. Application potential has been evaluated using in vitro cell differentiation tests, antioxidant capacity and anti-inflammatory properties. Moreover, the qualitative and quantitative analysis of lipid and polyphenolic extracts can be used as a chemometric tool for authenticity and place of origin testing.

Keywords: *Vaccinium*, berries, lipids, polyphenolics, application, valorisation, biorefining

Anotācija

Ogu sulas pārstrādes procesā veidojas būtiski atkritumu daudzumi – ogu spiedpaliekas, kas sastāv no ogu mizām un sēklām, un potenciāli satur vērtīgus savienojumus, kas varētu tikt izmantoti citās nozarēs. Pielietojot biorafinēšanas principus iespējams piešķirt pievienoto vērtību šiem atkritumproduktiem, iegūstot ekstraktus vai frakcijas ar specifisku pielietojumu. Šī promocijas darba mērķis bija izvērtēt lipīdu un polifenolu atgūšanas iespējas no ogu spiedpaliekām un veikt atgūto savienojumu izmantošanas iespēju izpēti. Ogu lipīdi, tai skaitā virsmas vaski, satur daudzus lipofīlus savienojumus ar dažādām funkcijām. Lipīdu ekstraktiem tika pierādīta antimikrobiālā aktivitāte un saules aizsardzības īpašības, atbalstot lipīdu ekstraktu un to frakciju pielietojumu kosmētikā. Tika optimizēta polifenolu un antociānu ekstrakcija izmantojot Atbildes virsmas metodi, kas ļauj iegūt maksimāli augstu ekstrakcijas iznākumu. Iegūtie, attīrītie polifenolu ekstrakti tika analītiski raksturoti un to izmantošana tika novērtēta lietojot dažādus *in vitro* šūnu testus. Iegūtie rezultāti par lipīdu un polifenolu sastāvu ogās un to spiedpaliekās var tikt izmantoti kā rīks, lai noteiktu ogu autentiskumu un izcelsmes vietu.

Atslēgas vārdi: *Vaccinium*, ogas, lipīdi, polifenoli, pielietojums, valorizācija, biorafinēšana

Contents

Abbreviations

ABTS – 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ACNS – Anthocyanidins, total anthocyanidins
BSTFA – N,O-Bis(trimethylsilyl)trifluoroacetamide
DPPH – 2,2-diphenyl-1-picrylhydrazyl
FRAP – Ferric reducing antioxidant power
GC-MS – Gas chromatography-mass spectrometry
HPLC-PDA – High performance liquid chromatography with photo diode array detector
ICPOES – Inductively coupled plasma optical emission spectrometry
IRMS – Light isotope ratio mass spectrometry
LOD – Limit of detection
ORAC – Oxygen radical absorbance capacity
PCA – Principal component analysis
ROS – Reactive oxygen species
RSM – Response Surface Methodology
SCO_2 – Carbon dioxide at supercritical conditions
SPF – Sun protection factor
TE – Trolox equivalent
TFA – Trifluoroacetic acid
TPC – Total polyphenolics
UPLC – Ultra performance liquid chromatography
UV/VIS – Ultraviolet / visible

Introduction

Waste as a by-product of industrial production and human consumption is one of the major challenges today. Food production loss and waste constitutes to ≈ 30 % of the waste, leading to losses of valuable resources, but at the same time creating environmental problems (FAO 2019). Existing food production, consumption and waste management approaches evidently do not correspond to sustainable development principles. The food waste and organic waste management problem becomes yet more urgent considering the aim to abandon fossil material-based production and promote bio-based economy – bioeconomy (EC 2018) and achieve climate neutral and resource saving development (Green Deal 2019). However, to achieve transition to bioeconomy much more knowledge and innovation is needed in respect to properties of materials and production, waste management and processing. The keywords related to progress of bioeconomy are biorefinery, valorisation and circular economy.

Berries, fruits and vegetables are amongst the most widely consumed foods and their processing is related to a production of major streams of waste. Food waste comes from the production of different products, such as juices, juice concentrates, canned and dehydrated fruits and berries, jams and others (Campos et al. 2020). For example, 366 million tons of apples per year are produced and their processing to juice leaves 3–4.2 million tons of apple press residues also called pomace (FAO 2019). Berries are becoming more and more popular and amongst them of are berries belonging to *Vaccinium* species. *Vaccinium* berries (cranberries (*Vaccinium oxycoccos* L.), American cranberries (*Vaccinium macrocarpon* L.), blueberries (*Vaccinium corymbosum* L.), bilberries (*Vaccinium myrtillus* L.), lingonberries (*Vaccinium vitis-idaea* L.) and bog bilberries (*Vaccinium uliginosum L.*) are a traditional element of diet in Latvia as well as other countries in NE Europe. Nowadays the interest about the phytochemical composition of *Vaccinium* berries has significantly increased, reflecting the interest of society in natural and healthy food and thus the interest in composition of berries and factors affecting beneficial health effects has also grown (Nile and Park 2014). Wild and cultivated *Vaccinium* berries are becoming commonly consumed products and thus the studies on their composition are expanding to improve existing and develop new applications of berries, their processing products and extracts. In this respect berry processing waste – berry press residues – are of especial interest as they contain high amounts of bioactive compounds with relevant chemical and nutritional value, such as lipids, polyphenolic compounds, fibre and others and can serve as an excellent source of valuable ingredients for food industries, health, cosmetics. From this perspective, not only the whole berry, but their press residue studies (extraction and phytochemical analysis) are of importance and are becoming more relevant considering needs of natural product industry. Despite significant achievements in the studies of *Vaccinium* berries, knowledge of their composition, processing of their wastes, considering the concepts of circular economy and berry waste biorefinery approaches, development of new fields of this processing waste application is not widely explored.

Aim of the work

The aim of the thesis is to study composition of *Vaccinium* spp. berries, common for NE Europe, as well as biorefining possibilities of their press residues to support development of extract applications in bioeconomy.

Hypothesis

To achieve aims of food processing waste utilization, methods of berry press residue valorisation and biorefinery should be elaborated, including in-depth study of obtained functional ingredient composition and their bioactivities to identify application potential in food supplements, cosmetics and authenticity testing.

Tasks of the work

1. Investigation of extraction, fractionation, purification and sample drying methods used for polyphenolic and lipid extracts prepared from whole berries and berry press residues.
2. Optimization of environmentally friendly extraction methods of biologically active substances from *Vaccinium* berries and their press residues according to biorefinery concept.
3. Development of polyphenolic extraction procedure from berry press residues using intensive extraction methods.
4. Characterization of the chemical composition of the studied berry polyphenolic and lipid extracts.
5. Investigation of prepared extract and extract fraction biologically relevant activities to support the use of berry functional ingredients for nutraceutical and cosmeceutical applications.

Scientific novelty

1. Development and optimization of extraction methods for polyphenolic extraction from *Vaccinium* spp. berries and their press residues.
2. Development of lipid extraction methods from *Vaccinium* spp. berries and their press residues.
3. In-depth analytical characterization of polyphenolics and lipids obtained from *Vaccinium* spp. berries and their press residues: identification and quantification of biologically active substances.
4. Elaboration of berry and their press residue extract biological activity guided fractionation methods.
5. Development of *Vaccinium* spp. berry and their extract authentication methods.

Applied significance of the study

1. Development of food waste (berry press residues) biorefinery approach: elaboration of environmentally friendly technologies for berry juice production waste processing.
2. Demonstration of beneficial uses for *Vaccinium* berry extracts and their fractions.
3. Development of berry extract application prototypes.

Approbation of the results

The results of the thesis have been published in 13 articles (*h*=9, 219 citations), in total author of the thesis has 25 scientific publications. The results have been presented in 18 international and local conferences.

Scientific publications related to the topic of the thesis

Articles in this list have been used for the preparation of thesis. Articles have been numbered accordingly to their topic (Figure 1.2.) and appearance in the Results section and are further referenced to as **Article 1**, **Article 2**, **Article 3** etc. throughout the text. All articles presented in this thesis are indexed in SCOPUS and Web of Science databases and belong to Q1 and Q2 fields of the respective issues. Full articles can be found by scanning the QR codes at the end of the thesis in section "Appendices – article depository" or by their respective DOI's found in the same section as well as in the above-mentioned scientific databases.

1. **L. Klavins**, J. Kviesis, I. Steinberga, L. Klavina (2016). Gas chromatography–mass spectrometry study of lipids in northern berries. *Agronomy Research*, 14 (2), 1328–1347.
2. **L. Klavins**, A. Viksna, J, Kviesis, M. Klavins (2019). Lipids of cultivated and wild *Vaccinium* spp. berries from Latvia. *FoodBalt 2019*, 198–203.
3. **L. Klavins**, M. Mezulis, V. Nikolajeva, M. Klavins (2021). Composition, sun protective and antimicrobial activity of lipophilic bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.) extract fractions. *LWT*, 138, 110784.
4. P. Trivedi, K. Karppinen, **L. Klavins**, J. Kviesis, P. Sundqvist, N. Nguyen, E. Heinonen, M. Klavins, L. Jaakola, J. Väänänen, J. Remes, H. Häggman (2019). Compositional and morphological analyses of wax in northern wild berry species. *Food Chemistry*, 295, 441–448.
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7. P. Trivedi, **L. Klavins**, A. L. Hykkerud, J. Kviesis, D. Elferts, I. Martinussen, M. Klavins, K. Karppinen, H. M. Häggman, L. Jaakola (2022). Temperature has a major effect on the cuticular wax composition of bilberry (*Vaccinium myrtillus* L.) fruit. *Frontiers in Plant Science*, 3497.
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11. L. Kunrade, R. Rembergs, K. Jekabsons, **L. Klavins**, M. Klavins, R. Muceniece, U. Riekstina (2020). Inhibition of NF-κB pathway in LPS-stimulated THP-1 monocytes and COX-2 activity *in vitro* by berry pomace extracts from five *Vaccinium* species. *Journal of Berry Research*, 10 (3), 381–396.
12. **L. Klavins**, E. P. Puzule, J. Kviesis, M. Klavins (2022). Optimisation of blueberry (*Vaccinium corymbosum* L.) press residue extraction using a combination of pectolytic enzyme and ultrasound treatments. *Journal of Berry Research*, 12(1), 41–57.
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Presentations and participation at conferences

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2. L. Klavina, L. Klavins, A. Huna, S. Strauta, M. Klavins (2016) Chemical composition of Bog Bilberries, blueberries and black crowberry. In: Abstracts of the 6th Global Summit on Medicinal and Aromatic Plants (GOSMAP-6), Riga, Latvia, 23.
3. M. Klavins, L. Klavina, A. Kukela, L. Klavins (2017) Berry press residues as a valuable source of polyphenolics: extraction optimisation and analysis. In: Abstracts of the 11th Baltic conference on Food Science and Technology "Food science and technology in a changing world", Jelgava, Latvia, 26.
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11. L. Klavins (2019) Solutions for developement of bioeconomics – use of food wastes to obtain products with added value. 10th International workshop on anthocyanins. San Michele all'Adige, Italy.

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13. L. Klavins, A. Viksna, J. Kviesis, M. Klavins (2019) Lipids of cultivated and wild *Vaccinium* spp. berries from Latvia. Abstracts of the "FOODBALT 2019 13th Baltic Conference on Food Science and Technology" Food, nutrition, well-being. Jelgava, Latvia, 20.
14. L. Klavins, J. Kviesis, M. Klavins (2019) Surface wax composition of wild and cultivated Northern berries. In: Abstracts of the 10th International conference "Biosystems Engineering", Tartu, Estonia 184.
15. L. Klavins, L. Saulite, R. Rembergs, K. Jekabsons, M. Klavins, R. Muceniece, U. Riekstina (2019) Characterization of five *Vaccinium* spp. berry pomace extracts, inhibition of NF-Kb pathway in LPS-induced THP-1 monocytes and COX-2 activity in vitro. Abstracts: 13th World Congress on Polyphenols Applications, Valletta, Malta, 95.
16. K. Saleniece, R. Rembergs, K. Jekabsons, L. Klavins, M. Klavins, U. Riekstina, R. Muceniece (2020) Anti-aging effects of five *Vaccinium* spp. berry pomace extracts in vitro. In: Abstracts of International conference on agriculture, biological and environmental sciences, Bonn, Germany, 646.
17. L. Klavins (2021) Valorisation of food wastes to obtain extracts with anti-oxidative and anti-inflammatory effects. 20th International conference on polyphenols – ICP2020. Turku, Finland.
18. L. Klavins (2022) Bioeconomy based biorefining solutions for valorisation of food wastes to obtain bioactive and functional ingredients. Conference of young scientists on energy and natural sciences issues – CYSENI, Kaunas, Lithuania, 641.

Protection of intellectual property

Patent granted on 14.01.2020 for the invention "Procedure for the extraction and purification of polyphenolics" (*in Latvian "Paņēmiens polifenolu iegūšanai un attīrīšanai"*). Patent Nr. 15504, granted in Latvia.

Contribution of the author in the development of thesis

Linards Klavins has done the preparation of sampling plans for various berries studied in this thesis, the gathering of the samples from sampling sites across Latvia as well as establishing network for sample gathering in other countries (Norway, Sweden, Finland, Lithuania), sample preparation for extraction, and analysis as well as different analysis themselves. Author has prepared elaborated investigation plans, including experimental design of conducted experiments, further participating in the analytical characterisation of samples, statistical analysis of obtained data, data visualisation and description.

Article 1: Conceptualisation 70%; Methodology 60%; Experimental work 80%; Data analysis 100%; Writing (original draft preparation, review, editing) 90%; Visualisation 100%.

Article 2: Conceptualisation 80%; Methodology 90%; Experimental work 90%; Data analysis 100%; Writing (original draft preparation, review, editing) 90%; Visualisation 100%.

Article 3: Conceptualisation 90%; Methodology 80%; Experimental work 60%; Data analysis 100%; Writing (original draft preparation, review, editing) 90%; Visualisation 100%.

Article 4: Conceptualisation 20%; Methodology 40%; Experimental work6%; Data analysis 50%; Writing (original draft preparation, review, editing) 30%; Visualisation 30%.

Article 5: Conceptualisation 90%; Methodology 100%; Experimental work 100%; Data analysis 100%; Writing (original draft preparation, review, editing) 90%; Visualisation 100%.

Article 6: Conceptualisation 20%; Methodology 50%; Experimental work 60%; Data analysis 50%; Writing (original draft preparation, review, editing) 30%; Visualisation 30%.

Article 7: Conceptualisation 40%; Methodology 60%; Experimental work 70%; Data analysis 70%; Writing (original draft preparation, review, editing) 30%; Visualisation 40%.

Article 8: Conceptualisation 70%; Methodology 80%; Experimental work 80%; Data analysis 100%; Writing (original draft preparation, review, editing) 90%; Visualisation 100%.

Article 9: Conceptualisation 90%; Methodology 90%; Experimental work 90%; Data analysis 100%; Writing (original draft preparation, review, editing) 90%; Visualisation 100%.

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Article 11: Conceptualisation 10%; Methodology 20%; Experimental work 40%; Data analysis 30%; Writing (original draft preparation, review, editing) 20%; Visualisation 30%.

Article 12: Conceptualisation 100%; Methodology 80%; Experimental work 60%; Data analysis 90%; Writing (original draft preparation, review, editing) 90%; Visualisation 90%.

Article 13: Conceptualisation 40%; Methodology 30%; Experimental work 40%; Data analysis 70%; Writing (original draft preparation, review, editing) 80%; Visualisation 60%.

Structure of the thesis

Due to the complexity of the studied material, each compound group of interest was studied accordingly to a pre-determined preliminary study plan (Figure 1.1).

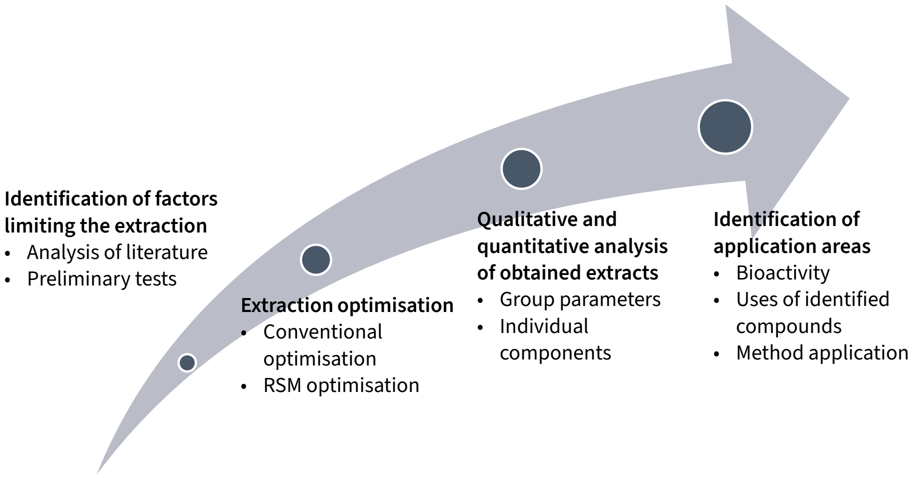
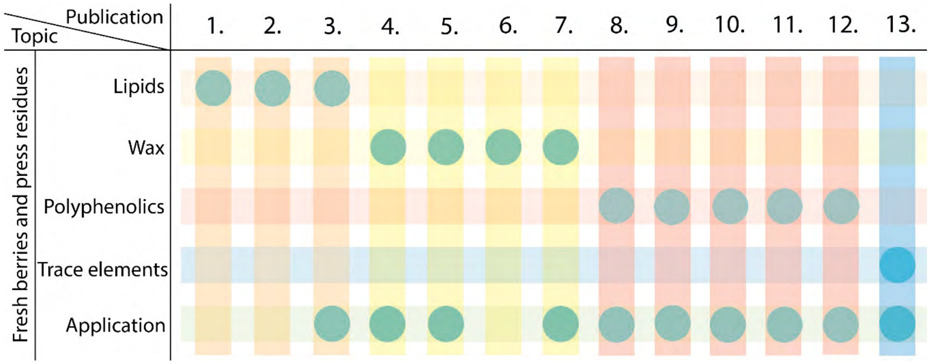


Figure 1.1. Preliminary structure for the scientific publication collections on lipids, waxes and polyphenolics of *Vaccinium* spp. berries.

The topics of the thesis have been divided into 5 subtopics which are represented by several published scientific articles. Articles 1–3 concentrate on berry lipids, 4–7 berry wax, 8–12 berry polyphenolics, 13 trace elements and stable isotope contents (Figure 1.2).



	Publication / Topic	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
Fresh berries and press residues	Lipids	●	●	●										
	Wax				●	●	●	●						
	Polyphenolics								●	●	●	●	●	
	Trace elements													●
	Application			●	●	●		●	●	●	●	●	●	●

Figure 1.2. Main topics of the published scientific articles within the scope of the dissertation.

The Results and Discussion (Section 3) has been built according to the structure presented in Figure 1.2 where the extraction, characterisation and application of each compound class have been highlighted.

1. LITERATURE REVIEW

1.1. Food waste problem and biorefinery as a tool to solve it

To achieve aim of the sustainable development, one of pre-conditions is rational use of resources, avoiding excessive formation of waste or developing technologies of their processing. Food waste is social, economic and environmental problem as from produced food ≈ 30 % is lost as waste, leading to losses of valuable resources, but at the same time creating environmental problems (FAO 2019). Also, in Latvia significant part of the produced food is lost as a waste (Tokareva and Eglite 2017), however, the losses during food production are not accounted. Existing food production, consumption and waste management approaches thus evidently do not correspond to sustainable development principles. The food waste and organic waste management problem becomes yet more urgent considering aim to abandon fossil material-based production and promote bio-based economy – bioeconomy (EC 2018), but to achieve optimal use of biological resources circular economy concept can be used. Circular economy is an efficient use of resources, applying green chemistry principles, leading to development of new business models and creation of innovative employment opportunities beyond other benefits (MacArthur 2013).

The major challenge to achieve transformation of the food production and processing approach is in the development of the new understanding of valuable ingredients in food and thus in food waste. Food processing logistics internally includes the concept of extraction of materials traditionally valued as food and thus only a part of the valuable material is consumed. Traditionally as the most important ingredients of the food are considered fats, carbohydrates and proteins, however, nowadays this concept is outdated. Also, food components, such as fibres, phenolics and many others are gaining value. Yet more, the impact of minor food components with positive impact on the human health is recognised. Terms such as functional food (food claimed to have an additional function related to health-promotion or disease prevention) or nutraceuticals (a broad umbrella term that is used to describe any product derived from food sources with extra health benefits in addition to the basic nutritional value found in foods) are becoming a trend in the food and health industries.

A total revision of the food waste concept is needed, and this problem is relevant at first in respect to fruit waste. Fruits are amongst the most widely consumed foods and their yearly production reach hundreds of millions of tons, but the amount of waste only in biggest producer countries reach 55 million tons (Wadhwa and Bakshi 2013). A large proportion of these wastes are dumped in landfills or rivers, causing environmental hazards. At the same time, fruit processing waste contains, for example, fruit seeds (contains proteins, lipids, nucleic acids and other valuable ingredients), fruit peels (contains polysaccharides, polyphenolics, alkaloids and others). However, to made fruit processing industry sustainable and implement circular economy principles there is a need: 1. to identify the valuable components of the fruit waste; 2. to develop innovative technological methodologies to change the flows of waste streams and recover valuable ingredients.

An approach which can address the fruit waste processing challenge is biorefinery. Biorefinery is a sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy. Biorefinery can provide multiple chemicals by fractioning an initial raw material (fruit and fruit waste biomass) into multiple intermediates (carbohydrates, proteins, triacylglycerols) that can be further converted into value-added products (Cherubini 2017; Pratima 2013). Substances obtained using biorefinery approach can be used directly, for example, as nutraceuticals, but also to replace synthetic chemicals (Table 1.1.).

Table 1.1. Application possibilities of fruit waste processing products.

Source	Processing waste	Product	Field of application	Reference
Blackberry	Pulp	Phenolics, xylitol	Nutraceutical	Dávila et al. 2017
Mango	Peels	Phenolics, tannin	Food industry	Rojas et al. 2018
Grapes, apples	Pomace	Bioethanol	Fuel	Cherubini 2017
Pineapple	Peel, stem	Bromelain	Biotechnology	Campos et al. 2019
Apples	Peel, stem	Pectin	Food industry	Virk and Sogi 2004
Citrus fruits	Pomace	Bioethanol, biogas	Fuel	Taghizadeh-Alisaraei et al. 2017
Grapes	Pomace	Fibres	Food industry	Deng et al. 2011
Melon	Seeds	Oil	Food	Mallek-Ayadi et al. 2018
Melon	Pulp, skin	Cucumisin	Dairy industry	Gagaoua et al. 2017
Aronia	Pomace	Phenolics	Biomedicine	Angelini et al. 2019
Fruits	Waste	Feed	Husbandry	Wadhwa and Bakshi 2013
Fruits	Seeds	Biodiesel	Fuel	Górnas and Rudzinska 2016
Tomato	Peel	Lycopene	Biomedicine	Ho et al. 2015
Bilberries	Pomace	Phenolics	Biomedicine	Ravi et al. 2018

Traditionally fruit waste is used for production of biofuel. Fruit biomass processing can help to replace oil with biomass as raw material for fuel and reduce the consumption of fossil fuels. In biorefinery, almost all the types of biomass feedstocks can be converted to different classes of biofuels (methane, biogas, bioethanol, hydrogen, biodiesel as well as solid biofuels, for example, biochar) applying elaborated conversion technologies (Cherubini 2017). Techno-economic and profitability analysis of food waste biorefinery to biofuels demonstrates profitability of this approach, at first considering possibilities to use known technologies and high requirements of the market (Cristóbal et al. 2018). At the same time from the plant biomass to produce biofuel dominantly carbohydrates are used and more complex compounds (for example, enzymes, alkaloids and others) during treatment process are destroyed. Also, the scale of the fuel production makes biofuel production from fruit wastes profitable only if significant amount of fruit wastes is available (as it is in case of pineapple, orange, mango and similar fruit processing).

Soil pollution is a global problem which is addressed in many developing and developed countries. Common soil pollutants include toxic compounds and chemicals, heavy metals, pathogenic fungus and bacteria (Mareddy et al. 2017). As the soil pollution has been a persistent problem in regions with intensive agricultural practices, many effective soil remediation strategies have been successfully developed and applied. Modern soil remediation practices enhance the overall quality of the soil by altering its structure, microbial communities and increase the fertility, as major factors in the used practices the environmental safety and low cost must be considered (Beesley et al. 2014; Maiti and Ahirwal 2019). At present, food and vegetable wastes are used as soil amendments to increase the concentration of organic matter and nutrients in the soil (Banerjee et al. 2017). Despite the benefits offered using fruit waste as soil amendments, care must be taken, in order to preserve the natural soil characteristics. Often food and vegetable waste are biologically unstable, they can contain microorganisms that could contaminate the soil and disrupt the native soil microbiome. Moreover, the pH level of fruit wastes can be acidic, therefore it is recommended to mix the fruit and vegetable waste with charcoal, wood ash or compost to reduce the possible impacts of low pH (Burgos et al. 2010; Ajila et al. 2012).

Fruit and vegetable wastes contain large number of valuable compounds; however, the processing of this material is limited due to various factors, including the transportation to the processing sites and microbial instability (Plazzotta et al. 2017). Processed fruit wastes contain residual moisture and very often are high in carbohydrates, which can lead to rapid microbial spoilage, rendering the potentially useful waste useless. To overcome the issue of fruit and vegetable waste "shelf-life", the produced waste can be dried and ground to flour, which can then be transported more easily and used for various purposes, for example, for use in food products, as soil amendments, for pharmaceutical applications, additives to animal feed (Santana et al. 2017; Roberta et al. 2014; Ferreira et al. 2015). Another investigated application of fruit and vegetable waste flours is the absorption of heavy metals, they are porous and fibrous which can increase the adsorption process and yield. Chemical compounds (pectins, cellulose, lignin) found in the flour contain hydroxyl and carboxylic groups, which could bind heavy metals, also proteins containing sulphur, phosphate, amino groups and polyphenolic substances can adsorb heavy metals (Ghimire et al. 2003; Meena et al. 2005). In case fruit and vegetable flour would be used as heavy metal sorbent, several factors should be considered – availability of the material used, environmental factors, concentration of heavy metals, pH of the used material., particle size (for the purpose of further clean-up), temperature (Pavan et al. 2006; Azouaou et al. 2008, 2010).

Evaluation of fruit waste utilization to develop new products with nutritional and functional properties (functional foods) for human consumption are being widely studied. Studies where fruit waste flours are used as main ingredient in healthy snack bars, bread, cookies, show that this refined by-product has the potential to be used as functional ingredient – the prepared food articles are rich in fibre, vitamins, minerals, protein, polyphenolics (Ferreira et al. 2015). Artificial flavourings, colour enhancers and other additives have been found harmful, when ingested regularly and in high doses, therefore the interest in the utilisation of natural food additives is increasing. Extracts prepared from agro food by products and waste are already used as healthier alternatives, for example, beetroot betalains, eggplant anthocyanins, tomato lycopene (Rizk et al. 2014; Faustino et al. 2019; Gengatharan et al. 2015). The added food waste extracts can act as flavouring, colouring agents, as oxidants, emulsifiers, firming, bulking and texturizing agents (Faustino et al. 2019). Fruit wastes contain skins and seeds of the plants, this type of biomass has residual carbohydrates and often are highly aromatic, the natural flavour contained within the fruit wastes allows this material to be used as part of juice, jams, as well as, for fermenting of alcoholic beverages (wine, beer, cider and distillation musts) (Majerska et al. 2019; Benvenutti et al. 2019). Certain fruit and vegetable wastes contain antioxidants in high concentrations, which can act as preservatives for food industry, considering this, shelf-life of meat products has been increased by addition of fruit or vegetable wastes. This approach increases the pathogen resistance (reduces their growth), retards oxidation, increases antioxidant capacity and nutritional value, when added to fish products, water retention has been increased and lipid oxidation reduced (Sánchez-Alonso et al. 2007; Lorenzo et al. 2017; Fernández-López et al. 2008).

Potential of fruit and vegetable waste use has been established as a safe and economically viable alternative to synthetic food additives. Presence of dietary fibre, enzymes, sugars, polyphenols, minerals, oils and vitamins in this biomass indicates the possible utilisation potential in different industries (food, cosmetics, agricultural, dairy). Noteworthy is the fact that many of the substance groups found in fruit and vegetable wastes have proven health benefits, which is an attractive trait in production of functional foods. In order to prepare functional ingredients from waste biomass it is important to improve the drying, storage and extraction techniques used. Selective extraction procedures should be developed to improve extraction yield and improve purity of the final product. Circular economy approaches should also be taken into account when developing such methodologies – local fruit and vegetable wastes, which are cultivated or grown in the wild in specific geographical regions, should be processed close to the origin of the biomass, therefore reducing the costs and increasing the socio-economic benefits of biorefinery of fruit waste and by-products.

The biorefinery concept can be oriented towards recovery of added value components with diverse application potential. The aim of the biorefinery process accordingly to this approach is to obtain maximally full spectra of substances or groups of substances with an application potential in bioeconomy. Thus, such biorefinery concept fully corresponds to bioeconomy concept and is aimed at the full use of substances present in fruit biomass. The direction of total biorefinery process can start with the isolation of most labile compounds (usually enzymes and other proteins), followed by alkaloids, phenolics and lipids, and ending up with more stable compounds, such as polysaccharides and fibres (Banarjee et al. 2017). However, this biorefinery strategy can be considered as more or less theoretical concept, as attempt to isolate everything most probably will not be feasible from the perspective of real production economics (Bilal et al. 2020). Still demonstration of recovery of possibly highest number of valuable components from fruits and fruit waste is exciting demonstration of power of biorefinery concept. Another biorefinery strategy concentrates on the isolation of substances considering their market potential and economically most rational isolation (extraction) technology. Accordingly to this concept the attention is focused on the isolation of one or few components from a fruit/fruit waste biomass, but the waste-of-waste is finally processed to fuel (usually bioethanol) or used as feed or composted.

Presently this approach is the most popular one and the groups of substances of interest with higher added value and application potential are enzymes (bromelain, cucumisin, antifungal peptides and others) (Campos et al. 2019; Gagaoua et al. 2017; Pelegrini et al. 2006), antioxidants (different polyphenolic compounds) (Fernández-Ponce et al. 2012; Lorenzo et al. 2018; Angelini et al. 2019; Ravi et al. 2018), pectin (Virk and Sogi 2004; Maran et al. 2014), oils and fats for application in health care, cosmetics and as food (Gaur et al. 2004; Mandawgade and Patravale 2008; Ho et al. 2015) essential oils (Yu et al. 2007) and other groups of substances.

Amongst key factors affecting the development of biorefinery methods and implementation of biorefinery technologies is the impact of the process on the environment (Bilal et al. 2020). Considering this, the recent developments consider use of environmentally friendly extraction methods, for example, use of extraction with supercritical fluids (Lizcano et al. 2019; Yu et al. 2007), use of low-toxicity solvents (Xu et al. 2016; Mohtar et al. 2017), innovative biomass treatment methods (Ho et al. 2015; Ravi et al. 2018), enzyme assisted extraction (Maier et al. 2008) and other methods.

1.2. Wild and cultivated berries in bioeconomy of Latvia

An approach to address the ecological, environmental, energy, food supply and natural resource challenges that Europe and the world are facing is bioeconomy. Bioeconomy is "the production of renewable biological resources and the conversion of these resources and waste streams into value added products, such as food, feed, bio-based products and bioenergy. Its sectors and industries have strong innovation potential due to their use of a wide range of sciences, enabling and industrial technologies, along with local and tactic knowledge." (Innovating for Sustainable Growth – A Bioeconomy for Europe" 2012). Innovative biological and technological research and development of new methods can support both intensive and still sustainable production. Use of biomass and its processing can change the industrial resource base and contribute at the development of climate neutral society as well as to conserve the Earth's non-renewable resources. Thus, the bioeconomy offers an opportunity to reconcile economic growth with environmentally responsible action and is one of EU strategic aims of development (A sustainable bioeconomy for Europe 2018).

Development of bioeconomy is identified as one of the strategic targets of the sustainable development of Latvia (National Strategy of Bioeconomy 2030). The National Bioeconomy Strategy calls for a bioeconomy as a key element for smart and green growth, that will allow to improve the management of renewable biological resources and to open new and diversified markets in food and bio-based products. Establishing a bioeconomy in Latvia holds a great potential as it can maintain and create economic growth and jobs in rural, coastal and industrial areas, reduce fossil fuel dependence and improve the economic and environmental sustainability.

The key for success of bioeconomy is to develop new biorefining technologies to sustainably transform renewable natural resources into bio-based products, materials and fuels. To achieve the aims of bioeconomy research and innovation is needed (van Lancker et al. 2016), supporting creation of new knowledge, new products, new technologies as well as replacing fossil material-based products with biomaterial-based ones.

A prospective product for development of bioeconomy are berries (Berry Fruit… 2007). Berries are highly valued, considering their appearance, taste and nutritive properties and health benefits of their consumption. Such berries as grapes are cultivated for thousands of years and their consumption and processing by itself is an industry, but also berries, common in Northern regions, such as cranberries are cultivated in significant amounts. For development of bioeconomy of Latvia of especial value are berries belonging to *Vaccinium* spp. These berries include bilberries, lingonberries, bog cranberries, bog bilberries, American cranberries, blueberries and others. These berries are native in Latvian forests and bogs or they are cultivated successfully in local farms. Traditionally these berries are consumed fresh or made into jams, more recently a variety of products containing *Vaccinium* berries are developed and sold – juices, dairy products, cosmetic products, functional foods, food supplements and others. The processing of fresh berries into different refined products inevitably leads to production of fruit waste. The ability to offer a complete refinery of a particular biomass could be solved by biorefinery approaches. Due to the potential of *Vaccinium* species berries, their polyphenolic and lipid contents, local production and wide availability, these plant species should be evaluated for their prospective biorefinery solutions in order to promote practices of bioeconomy (Figure 1.3).

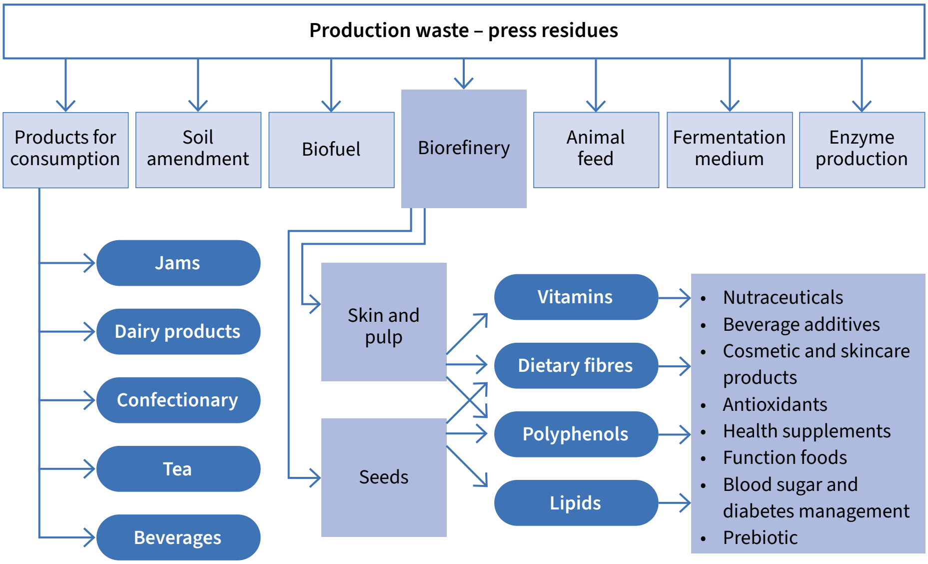


Figure 1.3. *Vaccinium* berry press residue utilisation potential.

Several examples exist where berry press residues (or other types of berry wastes) are used as starting point for refining of valuable, natural substances. Consumer demand for natural, instead of synthetic, food components is driving market for several plant-based food additives. Various extraction methods are being tested in lab- and pilot-scale plants in order to retrieve functional or bioactive ingredients or use berry pomace as food ingredient. Blueberry wine pomace has been processed using ultrasound-assisted

Table 1.2. Berry pomace used for the extraction of specific groups of compounds.

Type of waste	Compound of interest	Reference
Bayberry pomace	Anthocyanins, flavonols	Zhou et al. 2009
Chokeberry pomace	Flavanoids	Vauchel et al. 2015
Black currant pomace	Total polyphenols, anthocyanins	Galvan D'Alessandro et al. 2014
Blueberry pomace	Total polyphenols, anthocyanins	Paes et al. 2014
Blueberry and cranberry powder	Procyanidins	Khanal et al. 2010
Blueberry pomace	Procyanidins	Khanal et al. 2009
Chokeberry pomace	Polyphenolics, procyanidins	Mayer-Miebach et al. 2012
Cranberry, raspberry pomace	Total polyphenols, phenolic acids	Laroze et al. 2010
Cranberry pomace	Quercetin	Raghavan and Richards 2007
Cranberry pomace	Proanthocyanidins	Roopchand et al. 2013
Cranberry pomace	Procyanidins	White et al. 2010a
Strawberry pomace	Ellagitannins, ellagic acid	Juskiewicz et al. 2015
Blueberry, blackberry pomace	Total polyphenolics	Salaheen et al. 2014
Blackcurrant, redcurrant, chokeberry, rowanberry, gooseberry pomace	Fats, total polyphenolics, fibre	Reißner et al. 2019
Chokeberry, black currant	Fibre	Quiles et al. 2018
Blackberry, blueberry pomace	Polyphenolics	Salaheen et al. 2016
Blackberry, blueberry pomace	Phenolic acids	Salaheen et al. 2017

extraction of anthocyanins, which has been demonstrated to be an efficient, economic and environmental extraction technology, at the same time reducing amount of produced waste (He et al. 2016; Bamba et al. 2018; Hu et al. 2019). Blueberry pomace extraction techniques are optimised using Response Surface Methodology (RSM) by Box-Behnken experiments and the obtained extracts are turned into free-flowing powders for improved shelf-life and easier handling (Meng et al. 2014). In another study use of blueberry pomace was investigated as potential source of fibre to be used in baked products (value added gluten-free cookies), antioxidative properties of blueberry pomace were also considered (Curutchet et al. 2019; Šarić et al. 2016). New extraction approaches are being developed, these methods do not use toxic solvents, they are economically feasible and fast, however, they must be tested and optimised for the specific biomass to be used for valorisation. Bilberry pomace has been extracted using microwave assisted hydro-diffusion with subsequent ultrasound and bead milling extraction for complete valorisation of the pomace – in this process a variety of polyphenolics and lipids were retrieved (Ravi et al. 2018). American and wild (bog) cranberries and their extracts are known for their use in the treatment and prevention of urinary tract infections (Jepson and Craig 2007), more recently the use of these extracts have been evaluated for food preservation. Cranberries are processed by the juice industry, which leads to the production of cranberry pomace. Extracts of cranberry pomace have been evaluated for their antimicrobial effects in minced pork, providing a safe alternative to synthetic preservatives and additives (Gniewosz and Stobnicka 2018; Stobnicka and Gniewosz 2018). Berry wastes are rich sources of various phytochemicals that can be either used as is or extracted for their valuable ingredients (Table 1.2.). As some of the berries in question are mass-produced, also the amount of waste available is considerable, therefore, the feasibility and prospects of berry waste valorisation must be considered.

1.3. *Vaccinium* spp. berries and their traditional uses

A natural resource found in the bogs and forests of Latvia are berries. Berries are traditionally used as food and in ethnomedicine. In the present day the use of wild and cultivated berries in food industry, biopharmacy, cosmetology is gaining more recognition and attention due to the proven health benefits of berry consumption. There are more than 450 species belonging to the *Vaccinium* species (*Ericaceae* family) (Abreu et al. 2014). Natural habitat of this genus is the Northern Hemisphere, however, approximately 40 species have been found native to the Neotropics (Mexico, Argentina, Guyana) (Lutein 2007). The most notable species of wild and cultivated berries associated with health benefits belong to the V*accinium* spp. (Nile and Park 2014; Skrovankova et al. 2015). The most recognized members of this genus are bilberries, blueberries, lingonberries and cranberries (Abreu et al. 2014). Approximately 36 *Vaccinium* species are used in ethnomedicine with more than 70 different claimed effects. The proposed effects are mainly connected with the digestive system, urinary tract diseases and endocrine/metabolic systems. The highest number of uses per species have been recorded for lingonberry, bilberry, and bog bilberry – the fruits of these plants (berries) are mostly used as food and medicals, while the use of leaves is entirely for medicinal purposes. The ethnobotanical uses of different plants are basis for establishing criteria for valid sources of potential investigations that could be conducted to find bioactive compounds (Abreu et al. 2014).

Vaccinium myrtillus – bilberry

Bilberry is a low-growing shrub (height 15–20 cm) that can be found across Northern Europe, North America and parts of Asia. This berry is often confused with blueberry, they are closely related, however, the berries of bilberry are smaller, and they have pigmented flesh. Bilberries can be found in coniferous and mixed forests and grows well in shade covering up to 100% of the forest floor. The berries are round, approximately 5–9 mm in diameter, they are dark purple to nearly black with whitish (glaucous) wax layer. Bilberries are considered one of the richest sources of anthocyanins in human diet (500 mg per 100 g of berries) (Zoratti et al. 2016). The high level of anthocyanins in bilberries is associated with its high antioxidative potential and other health benefits – most commonly bilberry consumption is known for improving vision. In Latvian traditional medicine leaves and fruits of bilberry plant are used (Rubine un Eniņa 2004; Ančevska 2020). Bilberry fruits are used to lower the blood-sugar levels, to treat and prevent acute and chronic gastrointestinal irregularities. Bilberry extracts have been used to stop hiccups and to treat vitamin C deficiency. Presently bilberries, especially those originating in Northern Europe, are associated with increased health benefits and a wide variety of blueberry containing food supplements are available. In consumer products bilberries are widely used due to their colour enhancing properties and mild, pleasant aroma.

Vaccinium oxycoccus – bog cranberry

Cranberries have had important roles in the traditional health and culture of indigenous people across Northern hemisphere (Brown et al. 2012; Abreu et al. 2014). Indigenous people of North America have consumed berries fresh, stewed with fish, eaten with meat, fresh or dried fruit were stored for winter in birch bark baskets underground (Brown et al. 2012). Bog cranberries were used to for treatment of mild nausea. Patches of berry plants have been considered the property of a family and passed through generations as fruit was picked in the fall, made into jelly or dried with other fruits (Moerman 2004). Drinks made of bog cranberries were common among the peasantry in Scandinavia as they do not require any sugar and it was a way to save the berries for the winter (Svanberg 2012). Also, in North of Russia wide use of bog cranberries was common (Лебедева и Ткаченко 2016; Belichenko et al. 2021). In Latvia ethnomedicine bog cranberries were used against colds (Ančevska 2020). Berries has been used also for treatment of skin disorders (dermatitis, irritations and others) (Mamedov et al. 2005) and in Northern Europe they have been considered of major importance considering diversity in applications and economical potential of berry use (Pieroni and Sõukand 2018).

Vaccinium macrocarpon – American cranberry

American cranberries are particularly popular due to their availability for consumption. In Latvia these berries are grown in cultivated bogs. American cranberry is an evergreen shrub with vines reaching up to 1 m in length. The berries are consumed fresh or candied, cranberry juice, due to its food preserving abilities is used as a natural additive to other juices and products. Extracts (also juice) of American cranberry are used as nutraceuticals in modern and traditional medicine in North America to treat and prevent urinary tract infections (UTI's) caused by *E.coli* (Shaheen and Noreen 2016). Ethnomedicine suggests the use of cranberry juice as a probiotic to avoid imbalance in the gut microbiome, however the major field of use is as a rich source of vitamins, at first – vitamin C.

Vaccinium vitis-idaea – lingonberry

Lingonberries can be found across whole Latvia, they grow in coniferous and mixed forests, bogs, felled forests, it is up to 25 cm high evergreen shrub with lifted, woody branches. Lingonberry flowers are bell-shaped, white to pale pink and produced in the early summer. The fruit is red berry with an acidic taste, ripening in early autumn. Lingonberry leaf tea is most often used in traditional medicine to treat gout, or as a diuretic agent to treat kidney stones (Ančevska 2020). Berries are used as an anti-inflammatory agent to relieve high fever and to increase appetite (Eniņa 2017). Lingonberries helped to inhibit mold growth as well as lingonberry extract (teas) strongly deterred the growth of bacteria that commonly cause food poisoning. Currently, lingonberries are mostly used in juices, jams and as anti-inflammatory dietary supplements.

Vaccinium corymbosum – blueberry

Similarly, to the American cranberry, also blueberries are cultivated. This species is native to the North America and in Latvia they are grown in raised bogs. Blueberries, depending on the variety, are 2–4 m high bushes, they blossom in May and can give harvest starting from end of June to late August. Due to the efforts put into the selective work to produce berries with favourable traits, the harvest yields are rather high, compared to other cultivated berries. Berries are mostly consumed fresh or to produce blueberry juice, which is later supplemented with bilberry juice to give the characteristic dark purple colour. Both leaves and berries are used in the traditional medicine to treat diarrhoea, atherosclerosis, eye diseases, diabetes (Shaheen and Noreen 2016).

Vaccinium uliginosum – bog bilberry

Bog bilberry or bog whortleberry is common in Europe, North America and Asia, it is a deciduous shrub that most often grows on the edge of peat bogs and forests with high humidity (rarely found in dense woodlands or shaded habitats) (Latti et al. 2010). The bog bilberry bush is usually 70–100 cm high, the flowers bloom in May with a pleasant, aromatic perfume. The flowering of these berries very often coincides with late spring frosts, therefore the amount of harvested berries is inconsistent from year-to-year. The leaves are oval, flowers are pendulous, urn-shaped, pale pink. The berries are oval with white flesh and can be distinguished by their almost whitish glaucous appearance (the wax layer of the berry), while the berry when touched reveals dark-blue skin (Jacquemart, 1996). Berries ripen in late summer – early autumn. Berries of bog bilberry have been used in the traditional medicine of Poland (Łuczaj and Szymański 2007), Japan (Masuoka et al. 2007), China (Li et al. 2011), Canada and Finland – they are an excellent source of vitamin C (Fediuk et al. 2002). In traditional medicine leaves and berries of bog bilberry are used to treat cystitis, gastritis, regulate intestine functions (Abreu et al. 2014). Bog bilberry is native to Northern Hemisphere and in Latvia can be found in bogs as it prefers acidic soils, however, they are less common than other *Vaccinium* species.

1.4. Chemical composition of *Vaccinium* berries

Consumption of berries is recommended by the dietary guidelines worldwide, whether they are fresh, frozen or processed into various products, consumption of berries provides the nutrients and phytochemicals for healthy diet (Bazzano 2005). The health industry has marketed the use of various food supplements and dietary ingredients as alternatives to fruit and berry consumption, fresh products are often seasonal therefore the use berry extracts is increasing. The health benefits associated with berry consumption are due to the high concentration of polyphenolics, antioxidants, minerals, vitamins and fibres (Strik 2007). Small fruits contain a large variety of phytochemicals – essential minerals, vitamins, phenolic acids, anthocyanins, flavonols, flavones, flavanols flavanones, which have been demonstrated to have different activities and functions within the human body (Moyer et al. 2002; Manach et al. 2004). Berries contain around 15% soluble solids (mainly carbohydrates – fructose) (Ramadan et al. 2008). The rest of the soluble matter is phytochemicals with especially wide diversity of polymerised (conjugated) polyphenolics with various degrees of oxidation and substitution patterns including glycolysation and other substituents (Seeram 2008).

1.4.1. Basic composition of *Vaccinium* berries

Functional *Vaccinium* berry components have received major attention, as they determine the berry application potential. Significantly less studied is the berry composition from perspective of their biochemistry and nutritional value. In general, *Vaccinium* spp. berries contain moderate amounts of carbohydrates, proteins and fibres with low calorific value, therefore berries of this species are considered dietic and recommended as part of weight-loss diets (Table 1.3). Essential and other amino acids have been studied in American cranberries (Dorofejeva et al. 2011) and concentrations as high as 2 g/100 g (dry weight) have been found with leucine and lysine at highest concentrations. Also, in blueberries 12–16 free amino acids were found (essential and non-essential) with arginine at the highest concentration, however, their concentrations much depends on the cultivar (Moon et al. 2013; Zhang et al. 2014; Song et al. 2014). During drying of berries, formation of furoylmethyl amino acids was noted (Michalska et al. 2018). Protein concentration in dried cranberries can reach 2.2 g/100 g (dry weight) (White et al. 2010a). Sequencing of several *Vaccinium* berries genome also has been done: American cranberries (Polashock et al. 2014), blueberries (Die and Rowland 2014), bilberries (Suvanto et al. 2020), lingonberries (Wang et al. 2017) indicating that there is general interest in the berries of this species and the genetic material has breeding and gene editing potential (Günther et al. 2020).

Table 1.3. Composition of general nutritional factors in *Vaccinium* spp. berries.

Nutrient	Unit	Bilberry[1]	American cranberry[2]	Blueberry[3]	Bog cranberry[4]	Lingonberry[5]	Bog bilberry[6]
Energy	kcal	51	44	57	46	53	52
Water	g	84	83	85	83	82	85
Carbohydrates	g	7.1	11.6	10	9.2	11.5	11.8
Proteins	g	0.7	0.4	0.7	0.4	0.8	0.6
Fat	g	0.6	0.1	0.3	0.2	1.2	0.4
Fibers	g	5.5	4.4	2.4	4.6	3.7	5.2

[1]Zoratti et al. 2016; [2]McKay and Blumberg 2007; [3] Zorenc et al. 2016; [4]Brown et al. 2012; [5]Bujor et al. 2018; [6]Colak et al. 2016

Nutritional value of fruits and vegetables depends on the growth conditions in the specific place of origin. Quality of nutrition for cultivated berries can be increased using nutrient and micro-element rich fertilisers, specific cultivar, choice of soil, watering practices and cultivation method used. Nutritional value of wild berries can differ depending on the place of origin, as has been showed in the case of bilberries (Zoratti et al. 2016) and the weather conditions (sunshine, precipitation, overall average temperature, sun radiation). Lack of studies where the nutritional value of less commercially important *Vaccinium* species have been studied, doesn't allow adequate comparison of the variability in these general factors.

1.4.2. Vitamins

Vitamins are a crucial component of a properly functioning human body, they help in maintaining physiological functions of the body, they reduce inflammation and boost the immune system. Some vitamins can also act as antioxidants and therefore help in relieving oxidative stress which can be the cause of chronic heart diseases, diabetes and cancer. Berries contain high levels of vitamins C, A, E and different types of vitamin B (Hakala et al. 2003; Skupien and Oszmianski 2004). The main vitamin found in berries and fruits, is the vitamin C – it is a natural antioxidant with various actions in human body, however, it must be noted that the levels of this vitamin largely depend on the species, variety, cultivation practices, ripeness and storage (Pantelidis et al. 2007). In cases where berries have been processed or dried the samples must be re-evaluated after the preparation process, as vitamin C is sensitive to oxidation and temperature degradation (Dorofejeva et al. 2011). For example, American cranberries contain vitamin A (3 μg/100 g fresh berries), vitamin C (12.6 mg/100 g), vitamin E (1.14 mg/100 g), lutein and zeaxanthin (86.45 μg/100 g), β-carotene (34.2 μg/100 g) (McKay and Blumberg 2007). Bilberries are reported to contain a variety of vitamin B types (B6 – 0.07 mg/100 g; B2 – 0.05 mg/100 g; B1 – 0.03 mg/100 g; B7 – 1.1 μg/100 g), vitamin A (5 μg/100 g), vitamin K (12 μg/100 g), vitamin E (1.9 mg/100 g), vitamin C (15 mg/100 g) (Zoratti et al. 2016). Although vitamins are highly regarded as an important group of substances that are essential for human metabolism, their concentrations, and contents in *Vaccinium* berries are not widely studied as the main compounds of interest, with most potential bioactivities are polyphenolics.

1.4.3. Macro- and trace elements

Berries contain both macro- and trace elements – concentration of these substances largely depend on the soil in which the plant has been growing as well as elemental composition of atmospheric precipitations. Main macroelements found in berries are potassium (K), magnesium (Mg), iron (Fe), copper (Cu), phosphorus (P), calcium (Ca), manganese (Mn), aluminium (Al) and sodium (Na). Compared to other fruits, some berries are also known for the ability to accumulate large quantities of specific elements, for example, iron, copper, sodium, aluminium (Chandler 1944; Rodushkin et al. 1999). In traditional medicine the use of *Vaccinium* berries in cases of increased menstrual bleeding is often mentioned, which is believed to re-new the iron reserves in the body. In human diet different minerals are involved in physiological and biochemical processes, for example, development of bones and teeth, they provide strength for the muscles, they act as crucial cofactors for enzyme activation/inactivation and regulate functions of hormones (Wach and Błażewicz-Woźniak 2012; Rohloff et al. 2015). Mineral composition of blueberries, cranberries and bilberries has been relatively more studied, in comparison with lingonberries and bog bilberries (Vollmannova et al. 2014; Karlsons et al. 2018; Dróżdż et al. 2018). Macro- and trace element concentrations in *Va*ccinium berries might also influence environmental pollution. For example, significantly elevated concentrations of trace elements in comparison with the background pollution sites were found in berries sampled in vicinity of ferrochrome and stainless-steel factories in Northern Finland (Pöykiö et al. 2005; Eeva et al. 2018). As a result of mining and metal processing industries, high concentrations of Ag, As, Be, Bi, Br, Cd, Hg, I, Ni, Pb, Sb, and Tl were found in berries in vicinity of mining areas in Northern Sweden (Rodushkin et al. 1999,). Recently, the impact of wood ash applications in forests on the elemental composition in berries has been studied and risks related to the increasing concentrations of trace elements has been found (Moilanen et al. 2006). In several studies has been tried to estimate metal concentrations in background sites as a reference values (Shotyk et al. 2019). Another aspect on the berry quality studies offers evaluation of element concentrations in edible products available on the market (Tahvonen and Kumpulainen 1991). As the growth conditions as well as metal accumulation patterns for different plant species vary, it is important to study contamination levels in species which are of importance for human consumption, as are all *Vaccinium* berries.

1.4.4. Carbohydrates

Carbohydrates are an important component of *Vaccinium* berries as they are core element of complex fibers and cell walls (structural carbohydrates) as well as mono- and disaccharides (non-structural carbohydrates) present in berry juices. However, complex studies of carbohydrate composition of *Vaccinium* berries have not received much attention and mostly it has concentrated on cranberries and bilberries, considering the amount of their production. Cranberry pomace solids have 35% insoluble fiber (USDA-ARS, 2004) and are rich with pectins which are largely responsible for cranberry and lingonberry sauce gelation (Salishcheva and Donya 2013). Bilberries contain 3.0 g/100 g dietary fibres, which is ≈ 25 % of their dry weight and they are composed of pectin, hemicellulose and cellulose, from which hemicellulose is mostly xyloglucan (Aura et al. 2015). Early investigations of the cell wall composition of *Vaccinium* berries revealed the presence of cellulose, pectin, and hemicellulose in berry pomace (Holmes and Rha 1978). Valuable component of *Vaccinium* berries are fibres and their amount in cranberries reach 65 g/100 g (dry weight) (White et al. 2010b) and in blueberries 26 g/100 g (dry weight) (Tagliani et al. 2019). More intensive utilization of fiber by-products has recently been the focus of some research (Zheng and Shetty 1998, 2000; Park and Zhao 2006; Raghavan and Richards 2007). The alcohol insoluble fraction of lyophilized berries is mostly protopectin (Stuckrath et al. 1998). The soluble fiber fraction of cranberries contains monomer units mostly of arabinose, glucose and galactose/rhamnose, with lesser amounts of xylose and mannose (30, 28, 21, 11, and 10% respectively) (Marlett and Vollendorf 1994). Although whole cranberry juice, like most juices, contains very little fiber (0.1%) (Cunningham et al. 2004), cranberry sauce contains substantial levels, ≈ 1%, of these insoluble carbohydrates (Marlett and Vollendorf 1994). In juice of bilberries dominate fructose and glucose (sucrose concentration was found to be very low, close to 0) at concentration ≈ 5 g/100 mL), but also myo-inositol as well as a number of other monosaccharides has been found (Sanz et al. 2004; Hilz et al. 2005). Glucose and fructose concentrations in bilberry juice depends on the growth conditions and other ecological factors (Casolo et al. 2020). Interesting data suggesting covalent interactions between complex carbohydrates and phenolics in cranberries (Zheng and Shetty 2000) are presented. Such interactions may improve the potential health benefits of *Vaccinium* berry carbohydrates as well as provide functionality to products derived from them. Also polysaccharide-protein conjugates (glycoproteins) and polysaccharide- lipid conjugates (i.e.

glycolipids) have been reported to possess important bioactive functions (antioxidant, immunomodulatory and anti-inflammatory activity). Soluble oligosaccharides are present at relatively high concentrations (≈ 20% w/w or greater) in many cranberry materials, and yet their possible contributions to biological activity have remained unrecognized (Coleman and Ferreira 2020). The cranberry xyloglucans that have been characterized to date contain a backbone of β-(1→4)-linked D-(+)-glucopyranose units that may or may not be substituted at O-6 with α-linked d-(+)-xylopyranosyl side chains (Coleman and Ferreira 2020). Arabinoxyloglucan oligosaccharides are present in cranberries and may contribute to the decrease of biofilm formation by uropathogenic *Escherichia coli* (Auker et al. 2012; Sun et al. 2015; Auker et al. 2019; Coleman and Ferreira 2020).

1.4.5. Lipids

Lipids are molecules, that are essential for plant and animal cells – lipids are responsible for the integrity of cell walls and organelles, they form a hydrophobic barrier, that protects and regulates cellular functions. Lipids can be identified as primary metabolites, as they are essential for plant growth and development, however, some of the lipid compounds can also be considered as secondary metabolites, as they serve functions that help the plants to better interact with the surroundings. Definition of lipids is largely generalised; they are biological substances with hydrophobic nature and in many cases soluble in organic solvents (Cassim et al. 2019). Numerous lipids contribute to cell metabolism regulation acting as signalling molecules. Lipids are the primary form of energy storage in plants and animals. The main form of energy storage in plants is glycerolipids, they are synthesised in the different organelles of plant cell and stored within the seeds or cell membranes. In the endoplasmic reticulum of epidermal cells, the fatty acids, instead of triacylglycerols, are transformed (alkane/alkene biosynthesis pathway) to cutin or wax constituents (alkanes, aldehydes, esters), which are deposited as the epicuticular wax on plant surfaces, to prevent water loss and protect the plant from other biotic and abiotic stresses. Due to the rapid development of analytical techniques, there are currently 45 648 unique lipid structures identified and classified (Murphy 2020). *In silico* databases contain more than 1 100 000 structures, belonging mostly to glycerolipids and glycerophospholipids. The advancements in the field of metabolomics have allowed the identification of more than 1116 lipid-related genes in human, 8500 genes, coding for 12500 proteins have been identified in other organisms. In the recent years, due to the quantity of novel structures, more attention has been focused on the classification of lipids, dividing them in eight categories – fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides (Horn and Chapman 2014). The available classification is regularly updated and improved, with addition or modification of sub-categories (Figure 1.4). *Vaccinium* spp. berries and their processing by-products are rich sources of lipids, they contain fatty acids, sterols, glycerolipids, which have positive effects on human health as functional compounds. Although most of the studies present on the *Vaccinium* berries concentrate on the analysis and biological activity of polyphenolics, lipids remain an important component of these berries with similar biological importance.

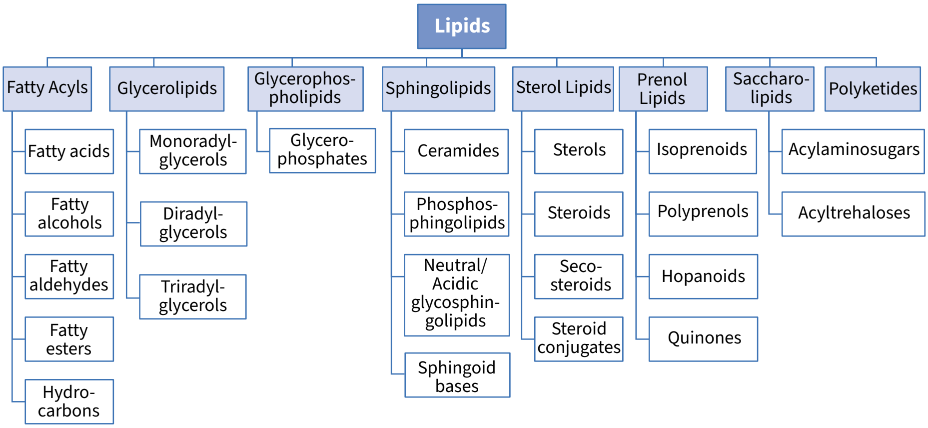


Figure 1.4. Lipid classification according to LIPID-MAPS classification system (Liebisch et al. 2020).

Fatty acids

Fatty acids are carboxylic acids consisting of an aliphatic hydrocarbon chain, which can either be saturated or unsaturated, with a terminal carboxyl group. Fatty acids of natural origin (plants) have an even number of carbon atoms. Most commonly fatty acids are found as a part of triglycerides; however, a small amount can also be found as free fatty acids. There are numerous uses of fatty acid in oleo chemistry and for food applications (Ahmad 2017). In human body fatty acids not only serve a function of energy storage, but they are also the buildings blocks of several types of tissue. Essential fatty acids, which are not synthesized by the human body can be divided into two groups, omega-3 and omega-6 fatty acids. Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA) are considered to be functional foods as they have various properties, for example, DHA is the main fatty acid found in the brain and eyes, it is responsible for neuronal functioning (Kaur et al. 2014). Berries of *Vaccinium* species are rich in a variety of omega-9, omega-3 and omega-6 unsaturated fatty acids as well as saturated C16 and C18 fatty acids (Gustinelli et al. 2018; Hurkova et al. 2019).

Sterols and isoprenoids

Isoprenoids are polycyclic compounds which are derived from the linear hydrocarbon squalene, these secondary plant metabolites are distributed throughout the plant kingdom. The C30 isoprenoids, also called triterpenoids are a subgroup of prenol lipids with high structural diversity. In plants triterpenoids are mainly found in a free form, however, they can also be found as conjugated esters – depending on the form present, triterpenoids can be found in different plant organs or cellular compartments (Hill and Connolly 2012). Triterpenoids have a variety of pharmacological activities, they display antimicrobial, hepatoprotective, anti-inflammatory, hypolipidemic, cholesterol lowering, anticarcinogenic properties and can act as anticoagulants (Patocka 2003; Petronelli et al. 2009). Triterpenoids are present in the fruit cuticles; therefore, consumption of fresh, non-processed foods is the main source of these phytochemicals. Berries contain a variety of triterpenoids, for example, alpha-amyrin, beta-amyrin, betulin, ursolic acid, oleanolic acid (Szakiel et al. 2012).

Sterols are monohydroxy alcohols with a typical backbone, consisting of four-ring structure. They are an essential part of every cell in eukaryotes and are responsible for control of membrane fluidity and permeability by interacting with other biomolecules within the membranes (Piironen et al. 2000). Sterols are found in their free form or as fatty acid esters. The most common sterol is cholesterol, which is a zoosterol. Plants, most commonly, contain a mixture of beta-sitosterol, campesterol and stigmasterol (phytosterols). Plant sterols and their derivatives, when consumed play an important role in the regulation of cholesterol absorption (Caballero 2005; de Jong 2003). Bilberries, lingonberries and other *Vaccinium* berries contain a variety of phytosterols, for example, sitosterol, diverse stigmasterol derivatives, lanosterol, citrostadienol and others (Yang et al. 2003; Koponen et al. 2001). Despite the functionality of sterols and isoprenoids in berries, there are only a few studies on these groups of compounds, concentrating on the most common forest berries – bilberries and lingonberries.

Wax constituents – aliphatic compounds, aldehydes, esters, alcohols

Cuticular wax layer protects the outermost plant surface, waxes cover the aerial parts of the plant – leaves, stems and fruits. This layer, consisting of a complex mixture of long-chain fatty acids and their derivates protects the plants from UV radiation, water loss and pathogen attacks (Kunst et al. 2003). The main components are the wax esters, consisting of a long-chain fatty acid and long chain alcohol, also alkanes, ketones, secondary alcohols, aldehydes and esters, secondary metabolites such as triterpenoids, sterols, and phenolic acids (Yeats and Rose 2013). These wax constituents are primarily a significant element of defence mechanism that protects the plant, they are highly hydrophobic and inert. Besides the secondary metabolites found in the plant wax (although in low concentrations), the hydrocarbon constituents have low biological activity. The most commonly used plant waxes are those extracted from carnauba wax palm (*Coernicia cerifera*) and candelilla (*Euphorbia cerifera*). Application of plant waxes include a variety of uses in hydrophobic coatings, varnishes, paints, candles, cosmetics. Waxes of *Vaccinium* berries are still an unexplored resource (Chu et al. 2018). The composition of these waxes could possibly allow their use in food articles, if solvent free extraction could be applied. Berry waxes contain triglycerides, sterols, triterpenoids and wax esters (Chu et al. 2017).

1.4.6. Polyphenolics

Polyphenolics are regarded as one of the most abundant groups of natural phytochemicals. Polyphenols consist of approximately 8000 individual substances (Leri et al. 2020) that consist starting from a single aromatic ring with one to several hydroxyl groups up to polymeric substances with molecular mass larger than 2500 Da (Rasouli et al. 2017). Based on the chemical structure of polyphenol aglycones they are classified as phenolic acids, flavonoids, phenolic alcohols, stilbenes, lignans. Sources of research being conducted on phenolic substances date back to at least the start of 19th century (Robiquet and Boutron 1837). The early studies mainly focused on the elucidation of the properties while structural characterisation is continuing, more and more research is conducted with emphasis on the biological activity of plant extracts containing polyphenolics or individual substances (Olivares-Vicente et al. 2018).

Table 1.4. Polyphenolic classes and their main food sources (Rasouli et al. 2017; Sharma et al. 2018).

Polyphenol class	Food sources
Flavonols	Black and green tea, red and white wine, nuts – almonds, walnuts, apple peel, blueberries, chocolate, broccoli, cabbage
Flavones	Different grains, vegetable oils, bell pepper
Flavanones	Tomatoes, citrus fruits
Flavanols	Black and green tea, hazelnuts, almonds, dark chocolate, blueberries, cranberries
Anthocyanins	Bilberries, elderberries, black currant, red wine, pomegranate
Isoflavones	Legumes
Proanthocyanidins	Bark of pine, bilberry, cranberry, grape seeds, red wine, tea, peanuts
Lignans	Whole bran cereals
Stilbenes	Grape derived products, lingonberry, cranberry
Phenolic acids	Spices, tea, berries

Phenolic compounds consist of a variety of molecules that have one or more aromatic ring – based on the number of phenol rings polyphenolics can be divided into several groups. The main groups of polyphenolics are phenolic acids, stilbenes, flavonoids and lignans (Figure 1.5). Formation of phenolic compounds happens *via* the shikimic acid pathway. Firstly, phenylalanine (amino acid) is formed, then the amino acid is catalysed by phenylalanine ammonia lyase releasing ammonia and transformed to *trans*-cinnamic acid which is a precursor of several phenolic acids and other phenolic compounds. *para*-Coumaric acid is formed from *trans*-cinnamic acid and can be further hydroxylated in positions 3' and 5' leading to formation of sinapic, ferulic or caffeic acids. These phenolic acids are termed phenylpropanoids. Flavanoids are formed when the phenylpropanoid compounds are condensed with malonyl coenzyme A, which produces chalcones that cyclize under acidic conditions (Chouhan et al. 2017).

Polyphenols are plant secondary metabolites therefore they have high physiological and morphological importance in plants. In plants polyphenols can have different roles, they can act as attractants for pollinators (reproductive role), defence against pathogens, contribute to plant pigmentation, antioxidants, UV-B protectants (Naczk and Shahidi 2006; Takahashi and Ohnishi 2004). These substances are almost exclusively found in plant material, with some exceptions, where plant polyphenols are metabolised and appear in animal organism as catabolites of dietary polyphenols (Table 1.4). Absorption of polyphenolic substances depends on the physical and chemical properties – configuration of the molecules, polarity (lipophilicity, hydrophilicity), molecular size. Aglycones are absorbed more easily than glycosides, the absorption can happen from small intestine to colon, during the digestion process the hydrophilic glycosides are converted into aglycones which are then absorbed (Williamson 2017). Main polyphenolic groups found in berries are flavonols, anthocyanins, flavan-3-ols, hydroxyl benzoic and hydroxycinnamic acids. In human diet polyphenolics are widely consumed, they have low toxicity and the consumed food items contain a large variety of polyphenolics (Lavefve et al. 2020). Concentrations of polyphenolics in fruits and vegetables can greatly differ depending on the environmental factors, for example, precipitation, exposure to sun, cultivation practices, yield of the plant and biochemical factors, such as, ripeness of the fruits, storage conditions, preparation method before the consumption (Miglio et al. 2007; Napolitano et al. 2004).

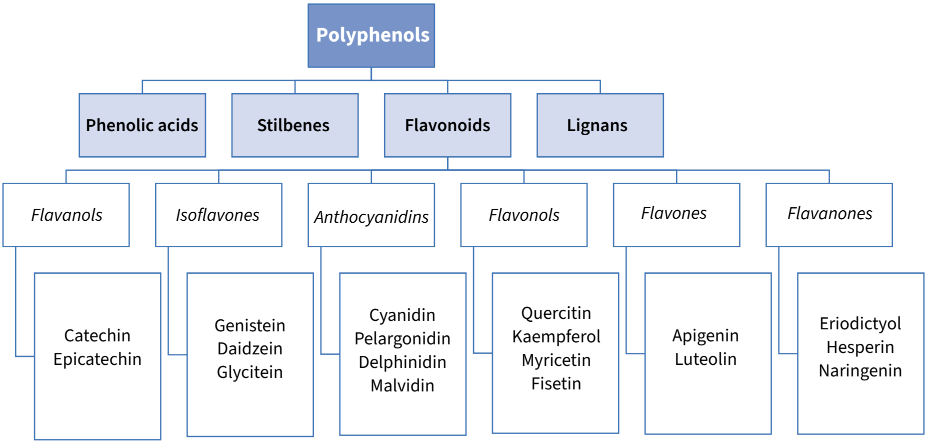


Figure 1.5. Polyphenolic groups, their classification, and examples.

Phenolic acids

Phenolic acids can be divided into two larger subgroups- hydroxybenzoic and hydroxycinnamic acids (Figure 1.6). The main hydroxybenzoic acids (C6-C1 structure) found in *Vaccinium* berries are vanillic acid, syringic acid, gallic acid and protocatechuic acid (Vinayagam et al. 2016). High concentration of gallic acid was found in bilberries, while protocatechuic acid was the predominant phenolic acid in lingonberries and cranberries (Colak et al. 2016; Hajazimi et al. 2016). The most abundant hydroxycinnamic acids (aromatic compounds with a three C side chain, C6-C3 structure) found in plants are *p*-coumaric acid, ferulic acid and caffeic acid. Blueberries and bilberries contain high levels of caffeic acid and derivatives, while lingonberries have low concentration of hydroxycinnamic acids (Määttä-Riihinen et al. 2004a; Määttä-Riihinen et al. 2004b). In plants these two subgroups of phenolic acids can be found in either free or bound forms. The bound forms are usually linked to a variety of plant metabolites through ester, ether or acetal bonds. Due to the variety of molecules phenolic acids can bind to, certain phenolic acids can be found in both, polar and non-polar plant extracts (Zadernowski et al. 2005).

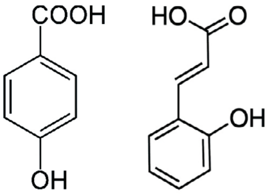


Figure 1.6. General structures of hydroxybenzoic and hydroxycinnamic acids.

Stilbenes and lignans

Stilbenes are a group of phenolics that can be found in a variety of plants. Stilbenes are most commonly associated with plant defence mechanisms and are produced within the plant after microbe attacks, injury or UV damage (Rivière et al. 2012). The basic structure of stilbenes is based on the C6-C2-C6 backbone, defined by two aromatic rings linked with ethylene bridge. Stilbenes can form structures with up to eight basic units (octamers) linked with various substituents, for example, isopropyl, methyl, hydroxyl or glycosyl groups. Currently approximately 400 stilbene derivatives have been identified. In human diet stilbenes are rarely present, there are only a few sources of stilbenes in the diet – grapes and grape derived products, peanuts, and *Vaccinium* spp. berries (Neveu et al. 2010). Resveratrol is the only known stilbene, to play an important role in human health. This molecule is believed to be somewhat responsible for the positive effects of Mediterranean diet, the properties include protection against many chronic diseases, including cancer, neurodegenerative and cardiovascular diseases (Sirerol et al. 2016; Reinisalo et al. 2015; Vang et al. 2011). Lingonberries have been found to have the highest concentration of resveratrol among *Vaccinium* berries (5884 ng/g dry berries) (Rimando et al. 2004).

Lignans are dimerised phenylpropanoids, where two aromatic rings are connected with a four-carbon bridge. Lignans are considered as phytoestrogens, in plants they are found as glycosides, however, after digestion in the intestine, the produced metabolites with estrogen (equol, enterodiol) like activity. Lignans are found in flaxseeds, sesame seeds, certain types of grains and vegetables. Certain lignans (lyoniside) can also be found in the stems and leaves of bilberries (Szakiel et al. 2011).

Flavonoids

The largest polyphenolic group is flavonoids, with currently identified 4000 individual flavonoids – its basic structure consists of flavan nucleus where 15 carbon atoms are arranged in three rings named A, B and C ring (C6-C3-C6 configuration) (Panche et al. 2016) (Figure 1.7). The aromatic rings A and B are derived from acetate/malonate and shikimate pathways, respectively. A and B rings are connected with a heterocyclic ring C. Various flavonoid subclasses are formed by different substitution (usually substitutions occur in ring C) and oxidation level of the basic backbone structure – naturally flavonoids occur as aglycones (basic structure) or as substitutions in the A and B rings (oxygenation, alkylation, glycosylation, acylation, sulphonation. These substitutions to the basic flavan nucleus result in the major flavonoid classes – flavonols, flavones, flavanones, isoflavones, flavanonols, flavanols and anthocyanidins. In general, flavonoids are

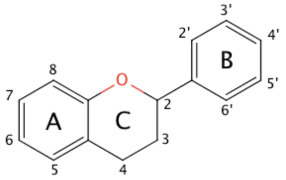


Figure 1.7. General structure of flavonoids.

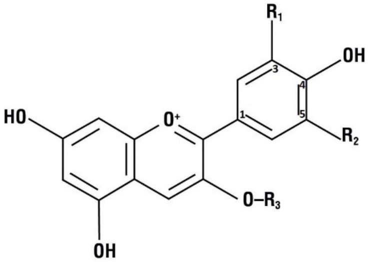
some of the most important antioxidants, which is due to their high red-ox potential. Some flavonoids can act as metal chelators (Grotewold 2006; Pietta 2000).

Anthocyanins

Anthocyanins are flavonoid subgroup that provide the characteristic colour (plant pigments) to plant tissue (roots, stems, leaves, fruits). The name *anthocyanins* come from the Greek words *Anthos* (flower) and *kyanos* (blue). The colour hue of these compounds ranges from red, blue, purple, to yellow and green depending on the pH level and the structural characteristics. At low pH the anthocyanidin molecule has positive charge (flavylium cation), by differing the pH of the environment the bonds and atoms are relocated thus changing the colour (Li et al. 2018). The group of anthocyanins consist of approximately 600 compounds and is considered to be one of the most important groups of flavonoids (Figure 1.8). In plants anthocyanins in highest concentrations are found in fruits and berries, for example, in bilberries, black currant, elder berries, also in vegetables, like red cabbage, red onion, radish. Naturally anthocyanins are found in the form of glycosides, where the unstable aglycones are stabilized by sugar or acyl moiety. There are approximately 30 different aglycones (anthocyanidins) however, approximately 90% of anthocyanins are made of cyanidin, pelargonidin, delphinidin, peonidin, petunidin, malvidin, which differ from one another in the methoxy or hydroxyl groups located on the B ring of the molecule.

In nature the main function of anthocyanins is the attraction of insects and other animals for pollination or seed dispersion. The localisation of anthocyanins in the upper part of the flower petals and epidermal cells has a physiological importance in the survival of plants. Also, the anthocyanin concentration in plant tissue can increase due to nutrient deficit, attacks of pests and pathogens, due to lowering of ambient temperature – in this context anthocyanins serve as adaptogens in order to overcome various stresses proposed by the environment, including drought, oxidative stress and lowering of the negative effects of UV radiation (Enaru et al. 2021).

Bilberries, black currants, elderberries, chokeberries are particularly rich in anthocyanins, both, the flesh and skin are strongly pigmented. Bilberry has been found to contain 15 different anthocyanins with the main anthocyanin being cyaniding-3-*O*-glucoside. The possible health benefits of anthocyanins have been studied in numerous research articles – this interest is based in the fact that anthocyanins act as strong radical scavengers and have potential in treatment and prevention of cardiovascular diseases, cancer, inhibition of virus proliferation and improvement of eyesight (Ockermann et al. 2021).

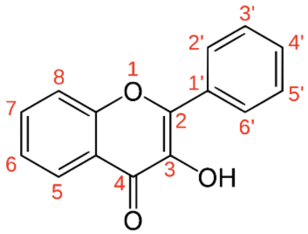


Anthocyanidins	**R_1**	**R_2**	**Λ_{max} , nm**
Pelargonidin	H	H	494 nm
Cyanidin	OH	H	506 nm
Peonidin	OMe	H	506 nm
Delphinidin	OH	OH	508 nm
Petunidin	OMe	OH	508 nm
Malvidin	OMe	OMe	510 nm

Figure 1.8. General structure of anthocyanidins.

Flavonols

Flavonols are characterised by a double bond on the C ring at the position 2–3 with a hydroxyl group at C-3. Flavonols are of special interest due to their antioxidative properties and biological activities which reach beyond antiradical scavenging. The powerful antioxidant effects are explained due to the additional presence of OH groups on the B ring and the double bonds conjugated with the keto group. The main representatives of flavonols are quercetin, kampferol, myricetin, isorhamnetin – they are typically found as glycosides with the sugar moiety available at the C-3 position (about 300 flavonol aglycones have been identified) (Figure 1.9). Also other sugar residues are commonly found in fruits and vegetables rich in flavonols, for example, galactose, rhamnose, arabinose, xylose, glucuronic acid. The sugar moieties are most commonly present as monoglycosides, however, also di- and tri-glycosides have been recorded. There are 200 quercetin and kaempferol glycosides described, which indicates the diversity of this flavonoid subgroup (Pietta et al. 2003). Flavonol contents can greatly differ depending on the seasonal changes, varietal differences, and also on more local factors, for example, the shading of the plant. The great differences that can occur within the same fruit in flavonol concentration are due to the biosynthesis of flavonoids stimulated by light – the leaves and part of peel that are in direct sunlight will have higher flavonol concentrations. Flavonols are known for their vibrant yellow colour and therefore have been used as wool and silk dyes since ancient times.



Common name	5	6	7	8	2’	3’	4’	5’	6’
Fisetin	H	H	OH	H	H	H	OH	OH	H
Quercetin	OH	H	OH	H	H	OH	OH	H	H
Myricetin	OH	H	OH	H	H	OH	OH	OH	H
Kaempferol	OH	H	OH	H	H	H	OH	H	H
Isorhamnetin	OH	H	OH	H	H	OCH_3	OH	H	H

Figure 1.9. General structure of flavonoids with representative compounds with their corresponding substitutions.

Flavanols

Flavanols or flavan-3-ols are a group of flavonoids that are hydroxylated at position 3 of the C ring. Catechins are the most prominent subgroup of flavanols – the members catechin, epicatechin, epicatechin gallate are the building blocks of tannins. Flavanols can be found as monomers, oligomers or even polymers (proanthocyanidins) (Dias et al. 2021). The oligomers and polymers consisting of epicatechin or catechin units are often referred to as procyanidins or condensed tannins – these molecules are responsible for the bitter and astringent character found in wines and chocolate. Also other flavanol aglycones can form oligomers, for example, gallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate. The polymerisation of catechins usually occurs through the formation of B-type bonds. High amounts of flavanols can be found in seeds and skins of vegetables and fruits, however, the intake of flavanols is limited as skins and seeds of fruits and vegetables are most often removed during processing and before consumption. Berries, however, are consumed whole, therefore the intake of flavanols and other flavonoids is not disrupted. For example, cranberries contain procyanidins, which are predominantly linked via A-type linkage, this type of structural feature is responsible for the anti-adhesion action against uropathogenic *E.coli*, that cause urinary tract inflammation (Dias et al. 2021). The high molecular weight, larger polymers (10 and more catechin units) are the major proanthocyanidins found in cranberries, the higher molecular weight is associated with better anti-microbial properties, while the oligomers of 10 and less units have increasingly higher anti-oxidative power, with monomers being the most active radical scavengers (Karak 2019).

1.5. Extraction of *Vaccinium* berries and study of berry extracts

A variety of methods have been developed for the preparation, extraction and purification of plant derived phytochemicals. The type of phytochemical extracted largely depends on the used extraction method, solvent and the optimisation of the two.

The most often used extraction techniques are: 1) solvent extraction; 2) pressurized liquid extraction; 3) ultrasound-assisted extraction; 4) microwave-assisted extraction; 5) supercritical fluid extraction in various setups. The mentioned extraction methods are often complemented with conventional liquid-liquid and liquid-solid extraction procedures to improve selectivity and the purity of the raw extract (Altemimi et al. 2017). In the extraction of polyphenolics, as well as lipids the extraction time, solvent composition, extraction intensity play an important role in order to prepare compound (compound group) specific separation protocols. In the case of polyphenolics, which are bound to the cell wall through ester or glycosidic linkage, intensive or enzyme-assisted extraction methods are needed (Tiwari et al. 2013). Polyphenolics once released from the cell-wall matrix become unstable and are prone to oxidation, therefore stabilizing agents/antioxidants must be added to the extraction medium. Alcoholic solvent systems consisting of methanol or ethanol are the most often used for extraction of various polyphenolic groups (Shi et al. 2005). Hexane and chloroform are the preferred solvents for extraction of lipids, however, due to the toxicity and hazards of these solvents the method of supercritical CO_2 extraction is recently preferred and by adding a polar or non-polar co-solvent to this extraction system it is possible to subsequently extract lipids and polyphenolics (Lorenzo et al. 2018). Characterisation of the extracts is done by spectrophotometry using various reaction reagents to produce specific colour reactions. Qualitative and quantitative analyses using chromatography with various detectors (MS, UV, VIS and others) are becoming available and the strategies for identification on new phytochemicals are developing rapidly.

1.5.1. Extraction of polyphenolics

An important group of substances of interest in *Vaccinium* berries are polyphenolics. Numerous procedures for the extraction of polyphenolics can be found using different solvents as media and extraction methods with varying intensity. Polyphenolics represent many groups of substances with significantly different solubility, so for their extraction polar solvents has been used: water, lowest alcohols, acetone or their mixtures, however, the most popular extraction system is aqueous ethanol, as it supports further use of extracts in food industry (Table 1.6). At the same time, different properties of polyphenolics allow to achieve aim of their group separation (fractionation) during extraction process (Ravi et al. 2018). Polyphenolics has been extracted from berries as well as from their pomaces and each type of berry biomass has its specifics, therefore the extraction procedure should be optimised based on: 1) the type of material used; 2) polyphenolic group of interest; 3) further use of prepared extract. In several studies the efficiency of extraction optimisation has been demonstrated (Ravi et al. 2018). Another factor influencing the extraction procedures from *Vaccinium* berries and their press residues is relatively low thermal stability of polyphenolic as well as their oxidation risks during treatment in presence of oxygen. Considering this the usual limits for the extraction temperature was 50–60 °C, but often to stabilise the phenolic structures, acidification was used. As in many cases polyphenolics are bound to cell membranes, thus, of importance are the use of intensive treatment methods (ultrasound assisted extraction, microwave hydro diffusion and others) as well as enzyme treatment and the number of studied approaches during last decade has increased (Tiwari et al. 2013; Dinkova et al. 2014; Ravi et al. 2018). Recently also green extraction methods – high voltage electrical discharges, pulsed electric field, and ultrasound-assisted extraction, were compared in terms of extraction yield of total and individual polyphenolic compounds, as well as the antioxidant capacity of berry extracts (Lončarić et al. 2020).

Table 1.6. Extraction methods and conditions of polyphenolics from *Vaccinium* berries and their pomaces.

Material	Extraction method	Extraction media	Reference
Bilberries	Ultrasound assisted, repeated extraction	2 stage extraction: 1. acetone, 2. acetone: water – 70:30	Aaby et al. 2013
Bilberry pomace	Stirring, repeated extraction	Water, 80 °C, 15 min	Aaby et al. 2013
Bilberry pomace	Microwave hydro diffusion, gravity; ultrasound assisted extraction	Ethanol 10–80% ,30 min	Ravi et al. 2018
Bilberries	Enzyme assisted extraction	Water, pH 3(HCl), 50 °C, 120 min	Dinkova et al. 2014
Blueberry pomace	Ultrasound assisted, repeated extraction	Acetone, 70 % acetone, citric acid, SO_2, 50–80 min, 50, 80 °C	Lee and Wrolstad 2004
Blueberry pomace	High voltage electrical discharges, pulsed electric field, ultrasound-assisted extraction	Ethanol, methanol 50% 1% HCl, 20–80 min	Lončarić et al. 2020
Lingonberries	Shaking	Ethanol 60%, 20 min 55 °C	Dróżdż et al. 2017
Bog bilberries	Maceration, repeated extraction	Water, 70 % methanol, 70% acetone	Colak et al. 2016
Cranberry pomace	Maceration	Water, 50% ethanol, pH 2–5, 80 °C, 120 min	Roopchand et al. 2013
Cranberry pomace	Soxhlet extraction, pressurized liquid extraction	Ethanol, water, 50–80 °C, 60–180 min	Tamkutė et al. 2020
Cranberries	Ultrasound assisted extraction, shaking	Ethanol:water:acetic acid, 70:30:1, 20 °C, 50 min	Tian et al. 2017

Polyphenolics are a large group of substances, due to the complexity of the molecules and possible carbohydrate, -acyl, -phenolic etc. moieties the identification is complex and requires advanced characterisation methods. To overcome the difficulties in identifying specific substances, methods that estimate the 'total' amount of the specific group or subgroup of polyphenolics have been developed. For analysis of total concentration of polyphenolics most widely Folin-Ciocalteu method has been used

(Folin and Ciocalteu 1927, Siriwoharn et al. 2004), which is continuously improved (Sánchez-Rangel et al. 2013). Also, for determination of total procyanidins (Payne et al. 2010), total anthocyanins (Lee et al. 2005), total flavonoids (Vongsak et al. 2013) relatively selective methods are offered. For identification and quantification of individual phenolics, liquid chromatography with different detection systems are used (Ignat et al. 2011, Lončarić et al. 2020).

1.5.2. Extraction of lipids

Extraction of lipophilic or low-polarity substances is usually performed using various non-polar solvents like hexane, petroleum ether, chloroform. However, as lipids are a large group of substances, also the solvents used for extraction as well as extraction conditions vary significantly. Berry seed oils can be obtained also using cold pressing (Parker et al. 2003). To increase the efficiency of lipid extraction heating (moderate), sonication with ultrasound or maceration is also used (Table 1.7). The sample used in the extraction has to be considered – the material has to be sufficiently dried and milled, especially in the case where seed-rich samples are extracted. While the efficiency of solvent extractions is comparably high, the environmental and work-safety aspects must be considered. Chloroform, for example, is a toxic solvent and it is known to cause mutations within human cells. As an alternative extraction using CO_2 in its supercritical state is proposed (SCO_2). The extraction using SCO_2 has similar miscibility

Table 1.7. Extraction methods of lipids from *Vaccinium* berries and their pomaces.

Material	Extraction method	Extraction media	Reference
Cranberry pomace	Supercritical CO_2	CO_2, 50 °C	Tamkutė et al. 2020
Cranberry seeds	Cold pressing	–	Parker et al. 2003
American cranberry	Solvent extraction	Boiling isopropanol, $CHCl_3$	Croteau and Fagerson 1969
Cranberry pomace	Supercritical CO_2	CO_2, 40–60 °C, 30–50 MPa	Kühn and Temelli 2017
Blueberry seeds	Cold pressing, solvent extraction	Methanol 100% Methanol:H_2O 80:20	Parry et al. 2005
Blueberries	Solvent extraction, Bligh&Dyer method	$CHCl_3$/Methanol 2:1	Wang et al. 1990
Bilberries	Supercritical CO_2	CO_2, 450 bar, 60 °C, 45 min	Jumaah et al. 2015
Bilberry seeds	Supercritical CO_2	80 min, 40–60 °C	Gustinelli et al. 2018
Bilberry seeds	Supercritical fluid extraction with chlorofluorocarbon solvent as a co-solvent	Norflurane, 8–10 bar, 30–45 °C	Cante et al. 2020
Lingonberries	Soxhlet extraction	Diethyl ether, 8 hrs	Szakiel et al. 2012

properties as hexane, by varying the extraction parameters (temperature and pressure) the extraction can be made more selective towards compounds/compound groups of interest. In addition, by using HPLC pumps, various solvents can be introduced into the SCO_2 extraction process allowing for simultaneous extraction of also polar compounds (Table 1.7). The extracts obtained do not contain any solvents (unless a co-solvent is used) as the CO_2 evaporates. Such system has low operational costs and low environmental impacts as the CO_2 used for the extraction process is re-circulated within a loop system.

For analysis of lipid components usually gas chromatography with different detection systems has been used (MS, MS/MS, HRMS and others) (Cajka and Fiehn 2014; Tranchida et al. 2007) and to increase volatility of lipids, protection of polar functional groups (-OH, -COOH) has been done (Topolewska et al. 2015). For lipophilic substances with higher molecular weight in form of ethers (for example, waxes and others) or esters (triglycerides and others), hydrolysis and/or transesterification before analysis has been suggested (Van Wychen and Laurens 2013).

1.6. Health benefits of *Vaccinium* berries and their extracts

"*You are what you eat*" – is a well-known idiom, which reflects the understanding of how important a healthy diet is and how it relates to overall well-being. The positive correlation between, what is considered a healthy diet (consisting of fruits, vegetables, fibre, meats high in unsaturated fats etc.) has been described and proven in numerous studies (Berry fruit… 2007, Nile and Park 2014; Bvenura and Sivakumar 2017). The relationship between the consumption of polyphenolic compounds and unsaturated fatty acids have been well established with reduced risks for heart disease, regulation of cell signalling pathways, fat transport, cholesterol synthesis antioxidative effect and others. The low toxicity of these substances makes them safe dietary elements for quality improvement of the food and pharmaceutical industry. Overall, the free radical scavenging flavonoids and the foods that are rich in these secondary metabolites can help humans to maintain healthier bodies by preventing the oxidative damage (Pappas and Schaich 2009, Shaheen and Noreen 2016.)

1.6.1. Free radical scavenging effects

The free radical scavenging (antioxidative) activity of polyphenols, among other biologically relevant functions, can be largely attributed to their chemical structures. Reactive oxygen species (ROS) and other free radicals are neutralized by the hydroxyl groups (electron or hydrogen donors) which are part of the conjugated aromatic features of polyphenolics. Prevention of oxidative stress is crucial as the ROS can irreversibly damage DNA, lipids or enzymes (proteins) that are responsible for vital functions in the organism. The mode of action of dietary polyphenolics is the ability to transfer hydrogen atom or an electron to reactive oxygen, nitrogen or chlorine species (Das and Roychoudhury 2014). The scavenging potential of polyphenolics depends on the number, degree and position of hydroxyl groups in the molecule.

Several methods exist for *in vitro* free radical scavenging potential evaluation – ferric reducing antioxidant power (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), however, none of these methods are relevant to physiological processes present in the human body, as the concentrations of antioxidants in human plasma is too low to be determined using these methods (Saint-Cricq de Gaulejac et al. 1999). This relates to the low bioavailability of polyphenolic compounds, therefore the concentrations necessary for relevant and measurable effects can be hard to achieve (Brglez Mojzer et al. 2016).

Free radical scavenging activity of *Vaccinium* berries has been widely studied and the main objectives have been: 1) development of activity during vegetation period as well as regional, interspecies differences in berries, 2) relations of individual substances or their groups to radical scavenging properties, 3) identification of relations between berry components, radical scavenging activity and biological/pharmacological activity (Yan et al. 2002; Määttä-Riihinen et al. 2004b; Määttä-Riihinen et al. 2005; Giovanelli and Buratti 2009; Caillet et al. 2011).

1.6.2. Cardiometabolic health

Consumption of polyphenolics from different sources, like coffee, tea, apples, chocolate have been related with reduced risks of cardiovascular diseases and type 2 diabetes (Hollman et al. 2010; Hooper et al. 2008; Liu et al. 2017; Shen et al. 2012). It is believed that the mode of action for polyphenolics related to reduction of cardiometabolic syndromes are regulation of glucose metabolism, altering of blood pressure, reducing inflammation, regulating of cholesterol levels in blood as well as the interaction with gut microbiome (Oteiza et al. 2018; Tomas-Barberan et al. 2016; Gonzalez-Sarrias et al. 2017). The Mediterranean diet, which consists of red wine, nuts, olive oil, herbs and other products that are rich in polyphenolics, has been shown to be correlated with reduced mortality connected to risks of cardiovascular diseases (Tresserra-Rimbau et al. 2014). However, results of such studies and trials should be interpreted carefully, as the consumption of fresh vegetables might have less impact on health than the consumption of less products with animal origin and the latter could contribute significantly to present apparent correlation with increased benefits of polyphenolics consumption.

Regular intake of foods and products rich in flavanols and procyanidins has proven the involvement of these molecules in reduction of cardiovascular-related outcomes (Greenberg 2015; Larsson et al. 2016). Increased intake of flavanols has been associated with 37% lower risk of cardiovascular diseases, 29% reduced risk of stroke and 31% reduced risk of developing type-2 diabetes (Buitrago-Lopez et al. 2011). Consumption of flavanols, depending on the study, can produce significant improvements in lipid metabolism and insulin resistance, at the same time lowering systemic inflammation (Lin et al. 2016).

Another group of flavonoids that have been observed to reduce risks of cardiovascular diseases are anthocyanins. Anthocyanins are metabolized by the microbiome to form metabolites with anti-inflammatory properties which can also have positive effects on the vascular health. In a study where anthocyanin rich supplements were introduced into the diet the lipid contents of blood and glycemic control improved for patients with untreated diabetes (Tang et al. 2017), also reduction in blood triglycerides and low density lipids have been observed (Liu et al. 2016).

1.6.3. Anti-tumor activity

Berries and their extracts have been tested for anti-cancer activity in different types of *in vitro* models dealing with specific tumorigenic processes (Marnett and Dubois 2002). Epidemiological studies suggest that diets rich in fruits, vegetables and polyphenolic rich products protect against formation of certain types of cancers (Vant-Veer et al. 2000). The effects of berry polyphenolics cover a large variety of tumorigenesis mechanisms – they can induce metabolising enzymes, modulate gene expression, modulate cell proliferation and apoptosis, interact with subcellular signalling pathways. Anthocyanins that can be found in high concentrations in bilberry (especially cyaniding-3-glucoside and cyaniding-3-rutinoside), ellagic acid, folic acid, beta-sitosterol, phenolic acids have been demonstrated to have anti-cancer activity against esophageal, colon, and different types of oral cancers (Johnson and Arjmandi 2013; Tulio et al. 2008; Boivin et al. 2007). Some groups of compounds have been proven to possess antimutagenic activity by blocking the metabolism of cancer cells, scavenging the free radicals and reactive oxygen species. Ellagitannins have been extensively studied for their anticancer and antimutagenic activity, especially ellagic acid, which is also found in *Vaccinium* berries (Gupta et al. 2019; Surh et al. 2000; Katsube et al. 2003). Plant extracts can contain several hundreds of bioactive phytochemicals, which can provide either antagonistic or synergistic effects. In the early stages of cell mutagenesis antioxidants can protect the cells, however, the mode of action for apoptosis is unclear, the effects of a single compound can be clear, however the effects of numerous bioactive compounds are hard to study due to the limitations of analytical equipment (complexity of unknown molecular structures) and costs for isolation of each of the compounds found in the plant extracts (Ceci et al. 2018; Tate et al. 2006; Liu et al. 2002).

1.6.4. Antimicrobial activity an interaction with microbiome

Polyphenolics can have either positive or negative modulatory effect on the composition of an individual's microbiome (Selma et al. 2009; Fogliano et al. 2011; Oteiza et al. 2018; Tomas-Barberan et al. 2016). Due to the low solubility of polyphenolic substances, they are often metabolized in the human gut to more available forms and metabolites with potential bioactivity (Raimondi et al. 2015; Tomas-Barberan et al. 2014). Certain polyphenolic substances can inhibit the growth of several microorganisms that are opportunistic pathogens or have an important role in healthy microbiome, for example *Helicobacter pylori*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimuium*, *Listeria monocytogenes* as well as viruses like hepatitis C, influenza, HIV and yeasts of *Candida* spp. (Duda-Chodak et al. 2015). On the other hand, certain polyphenolics have promoting growth effects of bacteria that are beneficial for the microbiome, for example, *Lactobacillu* spp., *Bifidobacterium* spp. (Gwiazdowska et al. 2012; Jin et al. 2012; Koutsos et al. 2017). In a study where anthocyanin rich blueberry probiotic was supplemented for six weeks the *Lactobacillus acidophilus* and *Bifidobacterium* spp. counts significantly increased when compared to the placebo (Vendrame et al. 2011). The immune system is largely dependent on the health of microbiome; therefore it can be assumed that polyphenolics have potential to maintain balanced and healthy microbiome which in turn can produce health benefits (Sampson et al. 2016; Ridaura et al. 2013; Masumoto et al. 2016).

Procyanidins from cranberries (*Vaccinium oxycoccus*) have been used to prevent urinary tract infections caused by the bacterium *Escherichia coli* attaching to the walls of urinary tract – cranberry extracts and juice have been used in both the traditional and modern medicine in the prevention of this type of bacterial infection (Howell et al. 2005; Kontiokari et al. 2001; Kontiokari et al. 2003). There are several modes of action that could determine the inhibition effect of berry phenolics – destabilization and permeabilization of cytoplasmic and plasma membrane, inhibition of extracellular microbial enzymes, changes caused to the microbial metabolism (Filiminova et al. 2018). Growth of human pathogens can be effectively inhibited by cranberry and bilberry extracts containing ellagitannins (Naczk and Shahidi 2004). Berry polyphenolics show antimicrobial activity against *Staphylococcus* spp., *Salmonella* spp., *Helicobacter* spp., and *Bacillus* spp. (Nohynek et al. 2006). Antimicrobial effects are primarily observed against Gram-negative bacteria, while Gram-positive bacteria can be inhibited only by specific substances (ellagitannins). Berries from genus *Rubus* (cloudberries and raspberries) have the highest antimicrobial effects observed, which is mainly due to the ellagitannin concentrations, growth of such human pathogens like *Klebsiella oxytoca* and *Proteus mirabilis* have been effectively inhibited by ellagitannin rich fractions (Puupponen-Pimia et al. 2001; Rauha et al. 2000; Chung et al. 1998). The effects of berry extracts on growth of different microorganism is not studied extensively – only the effect of cranberry procyanidins are well-documented. As berries and their extracts contain numerous polyphenolic substances, the isolation and identification of individual substances/groups of substances could reveal new antimicrobial agents (Aly et al. 2019; Filiminova et al. 2018; Satoh and Ishihara 2020).

1.6.5. Anti-inflammatory effects

Several studies suggest that in addition to the various health benefits provided by the increased consumption of berries and other fruits/vegetables also anti-inflammatory properties have been observed. Published results on blueberry effects indicate that extracts of this berry can block the activation of nuclear factor kβ and lower the expression of nitric oxide synthase-2, cyclooxygenase-2, IL-1β (interleukin-1β) and TNF-α (tumor necrosis factor-α) (Pomari et al. 2014). In another study, where the effect of gamma radiation was studied, it was showed that the cranberry extract was effective in increasing the superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) activity in liver, while reducing the TNF-α (Azab et al. 2014). Overall tests to prove anti-inflammatory effects are often criticised – very often the *in vitro* experiments are done using only single enzyme and no enzyme interactions are considered, which does not reflect the metabolism of a living organism (El-Baz et al. 2016). Even when mice model is used the inflammation processes are only evaluated using single dose of the testing material in high concentrations which very often shows positive effects on the inflammation markers, however, using this approach the sub-chronic doses cannot be determined and evaluated (Nardi et al. 2016).

2. MATERIALS AND METHODS

2.1. Plant material

Fresh berries and press residues for polyphenolic extraction

Berries harvested by hand in the summer and autumn seasons of 2016 to 2020 were used for the extraction of phenolic compounds. Bilberries and lingonberries were harvested in the forests surrounding Saulkrasti town located in the central Latvia, American cranberries and highbush blueberries were picked at a commercial farm (Strelnieki) on the outskirts of Jurmala City, and bog cranberries and bog bilberries were harvested in the bogs belonging to Kemeri National Park. More detailed berry origin and harvest times, depending on the experiments in question can be found in **Articles 1–13**.

Press residues were produced by freezing berries at –20 °C to improve the release of water and juice. Berries were then gently thawed at 5 °C. Once thawed, they were loaded into a hydraulic juice extractor (BioWin), draining all juice. Berry press cake was frozen once again at –20 °C to prepare it for lyophilisation. Once frozen, berries were freeze-dried for 3 days in a Labconco® FreeZone benchtop freeze dryer at –45 °C. Berry press residues used for the examination of the determined optimal anthocyanin/polyphenol extractions were dried at 40 °C to mimic the industrial drying process. Finally, dried berries were homogenised to a fine powder using an IKA® M20 analytical mill.

Berry press residues of blueberry and American cranberry were kindly donated by the juice producer 'VeryBerry' LTD.

Berries for lipid extraction

Two species of wild berries growing in Latvia were investigated for their lipid constituents: lingonberries (*Vaccinium vitis–idaea* L.) and bilberries (*Vaccinium myrtillus* L.).

Lingonberries were harvested in Varme, Latvia (56°54'25.6"N 22°15'25.2"E) in late August 2019, and bilberries were harvested in Kudra, Latvia (56°55'10.8"N 23°32'46.0"E) in mid–July 2019. After harvesting the berries were cleaned from leaves and other biological material to avoid any pollution. Berries were transported to the laboratory and within 5 hours post-harvest they were frozen to –20 °C. Analysis were conducted within 4 months.

Berries for wax extraction

Nine berry species common in Northern Europe were examined for their cuticular wax composition. Examined berries were- bog bilberry (*Vaccinium uliginosum* L.), bilberry (*Vaccinium myrtillus* L.), American cranberry (*Vaccinium macrocarpon*), lingonberry (*Vaccinium vitis-idaea* L.), black crowberry (*Empetrum nigrum* L.), gaultheria (*Gaultheria mucronata*), rowanberry (*Sorbus aucuparia* L.), hawthorn (*Crataegus alemanniensis*) and eight varieties of blueberry (*Vaccinium corymbosum* L.), namely, 'Blue crop', "Blue gold', 'Chandler', 'Chippewa', 'Duke', 'North blue', 'Patriot', 'Polaris'. The different blueberry varieties and American cranberries were harvested at a commercial blueberry farm Z/S "Strelnieki" located on the outskirts of town Jurmala, Latvia. Bog bilberries, bilberries, black crowberries and lingonberries were harvested from the forests belonging to Kemeru National Park. Rowanberries, hawthorn berries and gaultheria berries were harvested in the vicinity of town Saulkrasti, Latvia. To avoid contamination and possible damage to the outer layer of berries they were harvested into glass containers using metal forceps, both were previously washed with chloroform (≥99%, Sigma Aldrich, Germany). In total, approximately 700 berries of each species or variety were harvested, all berries were harvested in the summer/autumn of 2018. After the harvest berries were placed into a refrigerated sample box and delivered to the laboratory for immediate extraction of cuticular wax.

2.2. Extraction

2.2.1. Extraction of polyphenolics

Extraction optimisation

Optimisation of extraction of polyphenolic compounds and anthocyanins from American cranberry press residues in aqueous ethanol and methanol with TFA and formic acid was carried out using the Response Surface Methodology (RSM) (Myers et al. 2016). A two-factor and three-level central composite design consisting of eleven experimental runs (three replicates at the centre point) was employed. Possible effects of unexplained variability due to extrinsic factors in the two observed responses were minimised by randomising the run (experiment) order. The design variables were the concentration of ethanol/methanol (X_1, v/v %) and the concentration of TFA/formic acid (X_2, v/v %). The observed response variables were the total anthocyanins (mg/100g berry material) and total polyphenols (g/100g berry material).

Ultrasound assisted extraction

Extraction from cranberry press residues was optimised using ultrasound-assisted extraction, as specified in a previous study (**Article 8**). A dried/lyophilised and homogenised berry or their press residue sample (0.50 g) was weighed, and 50 mL of the respective solvent mixture was added. A 100W ultrasound bath (*Cole–Parmer)* was used for the experiments. After the ultrasound treatment, the samples were left shaking for 24h in the dark and then filtered to remove fine particles. Clear, filtered extracts were stored in dark at 4 °C.

2.2.2. Extraction of lipophilic substances

Extraction of cuticular wax

A modified method for extraction of cuticular wax was done using two extraction solvents, chloroform and a mixture of hexane/ethyl acetate (1:1) (≥99%, Sigma-Aldrich, Germany). Each species of berry was extracted three times using each of the solvent. In total, 6 replicates per berry species were prepared. For extraction, three 100 mL beakers were used. 50 mL of extraction solvent was poured into each beaker, which were previously cleaned with the same solvent. For each replicate a hundred berries were picked from the harvested sample and sequentially dipped one by one into the extraction solvent for 30 seconds in each of the three beakers containing the solvent. Clean metal forceps were used for the berry dipping. After the berry dipping, all of the contents of the three used beakers were filtered and combined into an evaporation flask. Each beaker was further washed twice with extraction solvent and added to the combined extract. Samples were evaporated under reduced pressure using Rota-Vap evaporator (Büchi, Germany). Samples were evaporated to approximately 5 mL and transferred to clean glass tubes. The remaining solvent was evaporated in a water bath (40 °C) (Cole Parmer, USA) under a gentle stream of nitrogen until dry. The dried berry cuticular wax samples were stored into a freezer (–20°C) until analysis.

Extraction of berry lipids

For the extraction of berry lipids 3 kg of selected berries were freeze dried and homogenised in a sample grinder (IKA, Germany). 120 g of homogenised berries were weighed in 1 L bottles with a cap and mixed with 600 mL of $CHCl_3$ then placed in an ultrasound bath for 20 min (Cole-Parmer, USA). The sonicated sample was then filtered through a paper filter. The used filter paper with berry particles was placed back into the extraction bottle and another 600mL of $CHCl_3$ were added. Filtration and re-extraction in ultrasound was repeated three times. The fourth extraction was performed by incubating the sample in $CHCl_3$ overnight at room temperature to increase the extraction yield. The water in the ultrasound bath was changed every 10 min to avoid evaporation of $CHCl_3$ and overheating.

After extraction all the extracts were filtered, combined and concentrated using rotary evaporator (Heidolph, Germany). After evaporation the berry lipids were dried under a stream of nitrogen (AGA, Latvia) and the dry samples were weighed and stored at 4 °C.

Supercritical CO_2 extraction

Berry or berry press residue extraction using supercritical CO_2 was performed with plant material that has been dried at 40 °C and milled to a powder (particle size <1 mm). 20 g of the dried sample were weighed into the extraction vessel (volume 100 mL). Separex (France) supercritical CO_2 extraction unit was used for the extraction with operating parameters set to 250 bar pressure, 50 °C temperature with a CO_2 flow rate of 0.4–0.5 L/min. Co-solvent (70% ethanol) was used to aid with the sample collection from the extraction unit separator. Extraction time was 1 hour.

2.3. Purification and fractionation of extracts

Fractionation of berry lipids

Fractionation was performed using 1600 mg of berry lipids. The weighed lipid sample (500–550 mg) was dissolved in 20 mL of hexane/chloroform mixture (1:1). To increase the solubility sample was sonicated for 10 min. After sonication the resulting lipid solution was mixed with 5g of KP–Sil silica gel (loading capacity >800 mg), air-dried and packed into the samplet.

The used KP-SIL 25g column was conditioned with 3 column volumes (CV) hexane. Elution of lipids was performed by using solvents of different polarity- 4 CV of each solvent was used in increasing order of polarity: hexane, hexane/chloroform (1:4 v/v), chloroform, ethyl acetate, ethyl acetate/methanol (1:1 v/v), and methanol.

The fractionation process was repeated three times, collected fractions of respective solvent were combined and concentrated using rotary evaporator and dried under a stream of nitrogen.

2.4. Analytical characterisation of polyphenolics

Determination of total anthocyanins

The spectrophotometric pH–differential method (Lee et al. 2005) was used to determine the total amount of anthocyanins in the prepared extracts. Two buffer solutions with different pH were prepared: 0.025 M potassium chloride solution with pH 1.0 and 0.4 M sodium acetate solution with pH 4.5; pH was adjusted with concentrated hydrochloric acid (HCl). Dilutions of the same sample were prepared using the buffers, so that the Abs at 520 nm would fall within 0.1–1.4 AU and would not exceed the 1:5 sample/buffer ratio. The diluted samples were left in dark for 20–30 minutes, and the measurements were made within 20–40 minutes. The absorbance of each dilution was measured at 520 nm and 700 nm against a deionised water blank using a Shimadzu UV-1800 UV-VIS spectrophotometer. The total content of anthocyanin was calculated using the following equation (Eq. 1):

$$\text{Anthocyanin content (cyanidin-3-glucoside eq., g/L)} = \frac{\mathrm{A} \times \mathrm{MW} \times \mathrm{DF}}{\varepsilon \times \mathrm{d}} \tag{1}$$

where A = (A520nm – A700nm) pH 1.0 – (A520nm – A700nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; d = cuvette path length in cm (1cm); ε = 26 900 molar absorption coefficient, for cyanidin-3-glucoside.

The extraction yield, g anthocyanin/100 g berry dry extract, was calculated using the following equation (Eq. 2):

$$\text{g anthocyanin/100 g berry dry extract} = \frac{Anthocyanin\ content(g/L) \times Volume\ of\ extract(L)}{Weighed\ sample\ weight\ (g)} \tag{2}$$

Determination of total polyphenols

The total polyphenols were quantified using the Folin–Ciocalteu photometric method (Siriwoharn et al. 2004). A calibration graph using gallic acid in the range of 0.050–0.350 g/mL was prepared (R^2=0.999). The standards were prepared by taking 1 mL of the respective gallic acid standard solution and mixing it with 5 mL of Folin–Ciocalteu reagent (1:10 dilution with water) and 4 mL of 7.5% sodium carbonate solution in water. Measurements were done at 765 nm after a 20–30 minutes incubation period at room temperature in the dark. Total polyphenols in g of gallic acid equivalents (GAE)/100 g berry dry extract were calculated using the following equation (Eq. 3):

$$\text{TPC g GAE/100 g berry dry extract} = \frac{C\ of gallic\ acid\ \left(\frac{g}{mL}\right) \times Volume\ of\ extract\ (mL)}{Weighed\ sample\ weight\ (g)} \times 100 \tag{3}$$

where C of gallic acid (g/mL) is calculated using the calibration curve regression equation.

Determination of anthocyanins using UPLC-PDA

Ultra–performance liquid chromatography (UPLC) identification and quantification analyses of anthocyanins were carried out using a Waters ACQUITY UPLC system equipped with a Quaternary Solvent Manager (QSM), a Sample Manager – Flow-through Needle (cooled to 4 °C) (SM–FTN), a column heater (CH–A) and a photo–diode array (PDA) λ detector. Data was collected using a Waters Empower software. Analyses were carried out at 35 °C using a C18 column (Acquity UPLC BEH C18 2.1×50 mm i.d., 1.7 µm) with a column pre–filter (Frit and Nut 0.2 µm, 2.1 mm). The mobile phase consisted of aqueous 5.0% formic acid (A) and methanol/1.0% formic acid in water (70:30 v/v) (B). The flow rate was 0.250 mL/min, and the gradient elution was from 80% to 75% of solvent A in 15 minutes, from 75% to 60% in 7 minutes, and from 60% to 0% in 18 minutes, followed by 10 min. of stabilisation at 80%. The total sample run time was 40 minutes, injection volume- 2.0 µL. Identity assignment was carried out considering the retention times. Anthocyanins were quantified using external calibration graph prepared from anthocyanin standard mixtures (3–100 mg/L; $R^2 > 0.9990$). The limit of detection (LOD) and limit of quantification (LOQ), respectively defined as a 3:1 and 10:1 peak-to-noise ratio, were 0.75 µg/mL and 2.20 µg/mL respectively.

2.5. Analytical characterisation of lipids

GC-MS samples of wax and lipids were prepared according to previously published methodology (**Article 1; 2**). Briefly, the extracted cuticular wax of each replicate was weighed (approximately 20 mg) into three separate GC vials, the sample was dissolved in 1300 µL pyridine (Sigma-Aldrich). Silylation was done using 200 µL N,O-bis (trimethylsilyl) trifluoroacetamide, BSTFA (Sigma-Aldrich), samples were heated for 1 hour at 60 °C. Total number of replicates ran on the GC system was 18 per analysed berry species. GC-MS analysis was performed using GC-2010 plus coupled with GC-MS QP-2010 Ultra mass detector (Shimadzu, Japan).

Extracted berry lipids, lipid fractions and waxes were weighed out (approx. 5 mg) in a GC vial and dissolved in 1.3 mL pyridine and 0.2 mL of BSTFA was added. Sample was then heated at 60 °C for 1 hour. The resulting sample was analysed using GC-MS (Table 2.1).

LabSolutions 4.30 software (Shimadzu, Japan) and NIST'17 (NIST, USA) Spectral Library was used for identification of compounds. The analyses were performed in triplicate.

Quantification was done by preparing standard solutions of heptadecanoate (≥99.0%), 1-dodecanal (≥98.0%), (±)-α-tocopherol (99%), 1-octadecanol (99%), and n-tetracosane (≥99.5%) (Sigma-Aldrich) in the concentration range 1.5–500 µg/mL. Quantification was done by firstly identifying the group of compounds the analyte belongs to and then the concentration was calculated using the respective standard.

Table 2.1. GC-MS programme and parameters used for qualitative and quantitative analysis of lipids and cuticular wax.

Parameter, settings	Cuticular wax analysis	Lipid analysis
Column	Restek Rxi® – 5MS	
Carrier	Helium	
Flow rate, mL/min	10.8	16.0
Gas pressure, kPa	70.0	77.8
Column flow, mL/min	0.71	1.18
Split ratio	1:10	1:10
Injector, °C	290	290
Injection volume, μL	1	1
Ion source, °C	230	200
Electron impact, eV	70	70
Interface, °C	290	250
GC programme	200°C for 2 min → 250 °C at 30 °C/min hold 7 min→ 310 °C at 10/min hold 14 min	75 °C for 2 min→ 130 °C at 30°C/min hold for 10 min→ 310 °C at 4 °C/min hold for 10 min

2.6. Assessment of biological and other activities

Determination of sun protection factor (SPF)

SPF values were determined according to a previously developed method (Mansur et al. 1986). In short, solutions at concentration 1.0 mg/mL of lipid fractions were prepared in chloroform. Absorption of each solution was measured in the range 290–320 nm every 1.0 nm three times using Shimadzu UV-1080 UV/VIS spectrophotometer. Before making the measurements baseline was corrected using blank chloroform. SPF was calculated using mathematical equation utilizing UV spectrophotometry (Eq. 4):

$$SPF_{spectroph,} = CF \cdot \sum_{290}^{320} EE(\lambda) \cdot I(\lambda) \cdot Abs(\lambda) \tag{4}$$

Where: EE (λ) – constant, erythemal effect spectrum (Sayre et al. 1979); I (λ) –solar simulator intensity spectrum; Abs (λ)- absorbance of measured sample; CF – correction factor (= 10).

Determination of antimicrobial activity

Extracted and fractionated lipids were tested for microbiological activity using Agar well diffusion method. Microorganisms obtained from the Microbial Strain Collection of Latvia (MSCL) were used- *Staphylococcus aureus* (MSCL 334), *Streptococcus pyogenes*

(MSCL 620), *Staphylococcus epidermidis* (MSCL 333), *Escherichia coli* (MSCL 332), *Proteus mirabilis* (MSCL 590) and *Pseudomonas aeruginosa* (MSCL 331). The inoculum of respective bacteria was prepared in sterile water with density of 0.16 at Abs_{520}. Miller-Hinton agar plates were inoculated with the prepared inoculum using Drigalski spatula and sterile tube, agar wells were prepared. The diameter of bed was 6 mm and the experiment was repeated two times. For the determination of microbiological activity, the lipid solutions were prepared in dimethyl sulfoxide (DMSO) at the concentration of 15 mg/mL. The solution was sonicated for 7 min. Before the solutions were added to agar wells it was put in warm water to dissolve lipids and then 70 µL of solution were added to each well. Agar plates were incubated at 37 °C, for 20 h.

In order to avoid solvent effects a control (blank) well containing DMSO solution was prepared. A solution of gentamicin (10 mg/mL) was used as positive control. The inhibition zones were measured after 20h of incubation.

Meteorological data

Bilberry cuticular wax analysis results were correlated with weather variables in each of the gathering locations. The closest weather stations to the bilberry collection sites (within a 50 km radius) provided the weather variable information. The meteorological information was gathered from weather stations in Oulu (Finland), Tromsø (Norway), and Riga (Latvia), by the VTT Technical Research Centre of Finland, NIBIO and Latvian Environment, Geology and Meteorology Center respectively. The information gathered included the following: eight weeks prior to harvest's average temperature (Tavg), maximum temperature (Tmax), minimum temperature (Tmin), and average precipitation (average) in millimetres (Pavg).

Data analysis and statistics

Quantitative data of cuticular wax composition was subjected to two-way analysis of variance (ANOVA) to evaluate the differences between the analysed berries, post-hoc Tukeys HSD was used to distinguish significantly different groups. Principal component analysis (PCA) on correlation matrix and hierarchical cluster analysis using Ward's method with standardized data was performed to evaluate relationship among various tested berries. Statistical analysis and data visualisation was done using SAS JMP®, Version 13 (SAS Institute Inc., USA).

3. RESULTS AND DISCUSSION

The following chapter will provide an overview of the main findings and proposed ideas elaborated within the course of this thesis as presented in scientific articles 1–13 (list of the publications in Introduction). Due to the complexity of the studied material, the thesis has been structured based on the compound groups of interest – lipids, waxes, polyphenolics, major, trace elements and light stable isotopes, detailed descriptions on the research done can be found in the sections 3.2, 3.3, 3.4 and 3.5, respectively. The research done in this thesis focuses on berry press residues (or pomace), however, to gain a better understanding of berries and their processing wastes, also whole, fresh berries have been investigated – this is due to the reason that berry press residues are a rather homogenous mixture of residual berry pulp, skins and seed, stems and sometimes also leaves, and thus, it is not possible to distinguish between, for example, lipids of seeds or lipids of skin or lipids of the pulp. By studying whole berries and berry press residues separately it is possible to identify the parts of the berries that could potentially contain beneficial compounds with potential application fields. The obtained results on whole berries could be further transferred and optimised to be used for berry press residues, for example, the seeds could be separated from the pomace biomass and thus only seed lipids could be extracted, or the skins could be separated for extraction of anthocyanins.

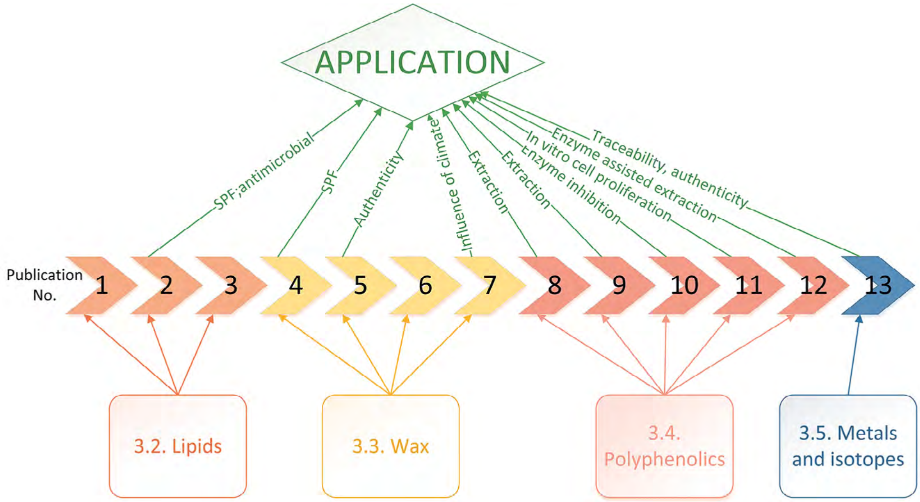


Figure 3.1. The relation between the themes of the published scientific articles on berry lipids (orange), berry wax (yellow), polyphenolics (red), metals and stable isotopes (blue) and their potential application areas (green).

The scientific articles presented in the thesis have been separated into five groups, each of the articles consists of introduction, where the reader is introduced to the background and the aim of the study, the used methodology is presented to the reader in a way that the experiments could be reproduced, the obtained data and its analysis is presented in the form of tables and graphs as well as discussion related to previous research done by other authors and explanations how the findings of the author of this thesis contribute to the field of phytochemical research and uses of bioeconomy based approach to biomass utilisation. To guide the reader Figure 3.1. provides a roadmap among the themes and specific studied potential application fields – each theme consists of a series of articles concentrating on extraction of the compound group of interest, the optimisation of extraction, chemical characterisation where group parameters are determined or specific compounds of interest are identified and quantified and finally the topicality of the obtained results is discussed in the relation to the possible applications in the bioeconomy field.

3.1. Berry press residue biorefinery strategy

There is now an alternative to the 100% oil economy: it is bioeconomy – a renewable agricultural resource-based production aimed at full use of biomass. Production and development of new products from biomass are based on biorefinery concept (Motola et al. 2018). Each constituent of the plant biomass can be processed, extracted and functionalized in order to produce non-food and food fractions, intermediate agro-industrial products and synthons (Lange et al. 2021) as well as energy. Bioeconomy should become a major supplier of a wide spectrum of value-added products including food and feed, bio-based chemicals, materials, health-promoting products and bio-based fuels thus addressing societal and consumer needs and contributing to climate change mitigation. The upgrading of side streams and leftovers from the collection and processing of biological resources is vital because it considerably increases resource efficiency, hence minimizing the carbon footprint, while also enhancing economic sustainability. Food waste, side-streams, and by-products from food processing can all be used to create new, high-value bio-based products by extending the useful life of existing resources. Using less fertilizer, pesticides, and water means less stress on the land and environment, which means more room and better habitats for wildlife, which means a reduction in biodiversity loss (EC 2020).

To achieve aims of bio-based economy, biorefinery plays a central role to achieve the progress as there is a need to develop of biorefinery systems supporting highly efficient and cost-effective processing of biomass of differing origin into bio-based products, and successful integration into existing infrastructure and already elaborated technologies (de Jong et al. 2012; Meyer 2017). Biorefinery is an approach when biomass is sequentially fractionated to get products with higher value, than the original biomass and/or energy, thus replacing fossil materials (Kamm and Kamm 2004). To reduce excessive use of fossil materials a key factor is the development of bio-based building blocks (chemicals and polymers), materials (fiber products, starch derivatives, etc) as well as pharmaceuticals from biomass (de Jong et al. 2012) at the same time replacing materials of fossil origin. Three major industrial domains can be concerned: molecules, materials and energy (Figure 3.2.) and as main products of biorefinery can be considered biofuels (bioethanol, biodiesel, biochar, biohydrogen and others), biopolymers and individual substances or groups of them (pectin, polyphenolics, proteins and others) which can be used directly or can serve as building blocks replacing substances of fossil origin. An important aim of the development of biorefinery approaches is reduction of greenhouse gas emissions.

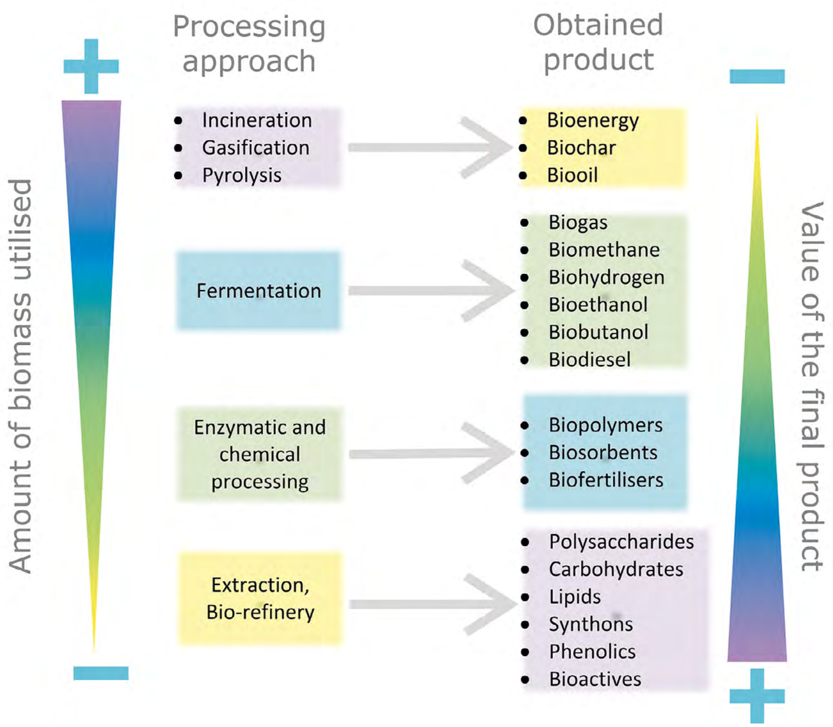


Figure 3.2. Biomass waste biorefinery strategies (modified from Stegmann et al. 2020).

As Figure 3.2. demonstrates, different biorefinery strategies allows to process differing types and amounts of biomass feedstocks at the same time producing products and energy, providing possibilities to fully replace fossil materials. The capacities to process biomass amount and the added value of obtained products significantly differs between different biorefinery strategies.

Biorefinery strategies are elaborated for several biomass types, but mostly for biomass processed in large scales and examples are wood biomass biorefinery (Octave and Thomas 2009), algae biomass (Balina 2020), orange press residue (Ortiz-Sanchez et al. 2021), agricultural lignocellulosic biomass (Clauser et al. 2021), and few others (Nayak and Bhushan 2019). Carbohydrates, lignin, proteins and lipids form ≈ 95% of plants, while other five percents are represented by vitamins, polyphenolics, dyes, flavors, alkaloids or other substances that are of high value, considering their direct application potential as a result of biorefinery. On the basis of these various plant components, different specific biorefineries can be outlined based on carbohydrates (monosaccharides, polysaccharides), lignocellulosic feedstocks and lipids as main sources of molecules (Octave and Thomas 2009). Considering the large volumes of biomass to be processed, in many cases well elaborated biorefinery strategies are available (Du et al. 2007; Pauly and Keegstra 2008; Chen and Dixon 2007).

A specific group of biomass waste are food wastes, as they are resulting from food production and are rich in substances of high nutritional value as well as contain other substances with possibly high application potential (Nayak and Bhushan 2019), for example, berry press residues (pomaces), produced as a result of berry juice production. So far berry, especially *Vaccinium* press residue biomass biorefinery strategies have not been in depth elaborated and proposed. The specific aspects to design *Vaccinium* berry press residue biorefinery strategy are:

1. Relatively small volume of biomass (*Vaccinium* berry press residues) prospective for biorefinery. Most widely from *Vaccinium* berries for juice production are used American cranberries and their world production in 2019 was 687 535 tons mainly by the USA, Canada, Chile, but in Europe it was significantly smaller and in Latvia, American cranberry production was 344 tons 2019 (World cranberry production … 2019). Berry juice production usually is decentralized and thus also the processing of the berry wastes (biorefinery) could be of relatively small size, smaller than any process aimed at processing of lignocellulosic or algae biomass.
2. Market value of groups of substances or individual ones obtained as a result of biorefinery process and their differences. The market for berry press residue processing products includes polyphenolics (groups of substances, such as, anthocyanins, procyanidins or individual substances, such as resveratrol and others). The other highly valued group of substances prospective for isolation from berry press residues includes lipids (waxes, fatty acids, sterols, terpenes etc.) and essential oils. The other groups of components of berry press residues which could be obtained using biorefinery are fibres, carbohydrates and polysaccharides, lignin do have significantly lower market value. Also, the value of energy possibly obtained using waste-to-energy approaches of *Vaccinium* berry press residue processing could be significantly lower. Thus, the biorefinery strategy should concentrate on the processing of most valuable components as the value of the remains after isolation of polyphenolics and lipids is significantly lower and their processing more expensive, supporting possibilities to use of utilization technologies, such as composting or integration of after-extraction wastes into processing technologies of other biomass streams.
3. High quality of *Vaccinium* berry press residues in comparison with other kinds of food processing wastes. Quality of food wastes is one of main problems in respect to their biorefinery as after the processing deterioration due to enzymatic hydrolysis, microbial contamination can start very fast. Situation of *Vaccinium* berry press residues is opposite: berries before juice production are cleaned and after juice extraction it is possible to dry the pomaces or freeze them to exclude quality deterioration. Thus, the biologically active components remain intact.
4. Level of knowledge on berry press residue, especially *Vaccinium* berry composition and possible biorefinery approaches. Despite results of recent studies on *Vaccinium* berry composition and few studies dedicated to studies of *Vaccinium* berry press residue composition, the knowledge needed to develop berry press residue biorefinery strategy is missing. Some proposals are elaborated for cranberry pomace biorefinery (Harrison et al. 2013). At first, there is no information on rational extraction

methods avoiding use to toxic and dangerous solvents as well as environmentally friendly extraction methods and further optimization of extraction methods needed to develop pilot scale or industrial production processes is not available.

The aim of the development of *Vaccinium* berry press residue biorefinery plan is to increase knowledge about valuable components of these berries as well as create efficient and modular biorefinery approach to gain the maximal benefit from this valuable biomass kind (Figure 3.3.).

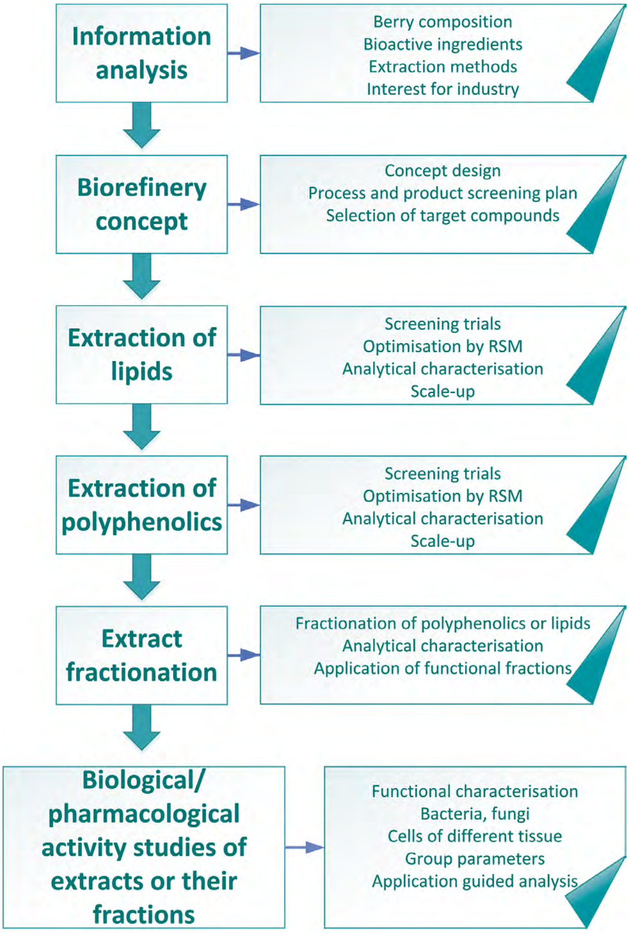


Figure 3.3. *Vaccinium* berry press residue biorefinery plan.

Vaccinium berry press residue biorefinery plan includes studies of the *Vaccinium* berry (cranberries, American cranberries, lingonberries, blueberries, bilberries, bog bilberries) and their press residue composition study, development of their extraction methods and optimization of it as well as testing of the isolated substances, groups of substances, to reveal their application potential and market value. The elaborated plan considers possibilities to realize it in a modular way (it means the realization of several stages of biorefinery, not only the full process) to obtain press residue components with highest application potential or interest for further industrial applications. The next main component of the biorefinery plan is as high environmental performance of the process as possible: 1) reduction of use of toxic chemicals replacing them with environmentally friendly ones, 2) energy and material saving methods during the waste processing, optimizing the material and substance flows, 3) optimization of the processes, at first extraction processes, 4) orientation towards groups of substances with similar properties as much as possible during the extraction process. Considering the aim to obtain components with highest added value, the utilization of the residues after extraction is not elaborated in details, but it can be suggested: 1) to use these residues for production of fibres, 2) composting, 3) thermochemical processing in hydrochar or biochar.

3.2. Berry and their press residue lipids

One of the main groups of substances in berries and their press residues are lipids. The research done on berry lipids includes sample preparation, extraction, analytical characterization and investigation of possible fields of application. Several tasks were set to reach interdisciplinarity throughout the research conducted on lipid compounds found in whole berries and berry press residues to investigate the possible uses in order to promote bioeconomy based solutions (Figure 3.4.).

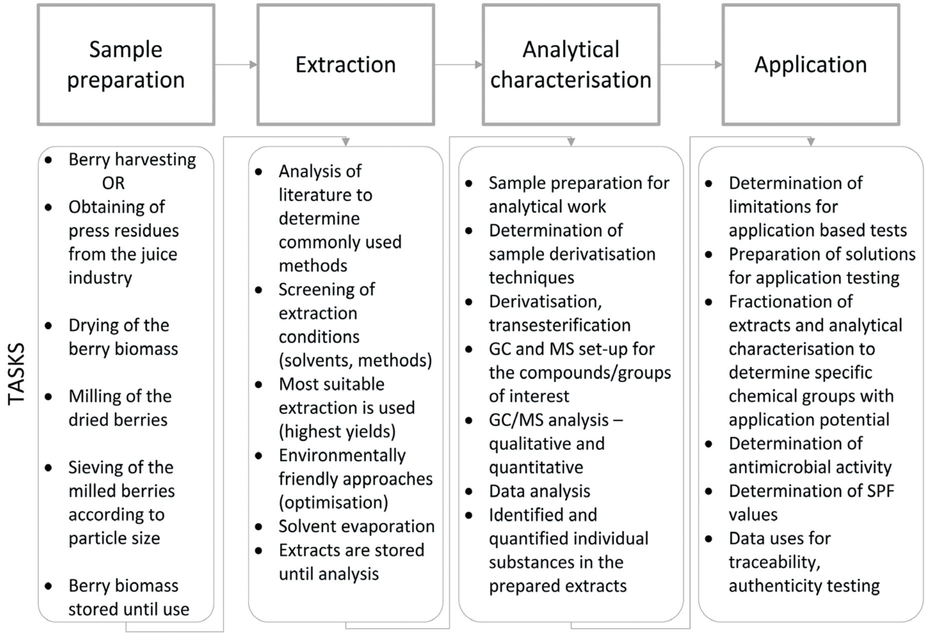


Figure 3.4. Berry biomass lipid research flowchart.

3.2.1. Extraction optimization of berry lipids

Lipids are functional compounds that are essential for any living organisms, they are considered as primary metabolites, since without lipids the functioning of an organism would not be possible. Lipids serve a variety of biological functions and their classification is rather complex – some classification systems are based on the function of lipids, some on the structural similarities. Another aspect in defining the compound class is much simpler – lipids are insoluble in water, but soluble in organic solvents. The investigation of berry lipids is based on the latter definition in combination with the most recent and most comprehensive classification system provided by LIPID-MAPS (Liebisch et al. 2020).

Berry lipids are a rather complex mixture of various compounds which span a wide range of substances. Using organic solvents, also other groups of compounds can be co-extracted, for example, carbohydrates, phenolic acids, which are not considered as lipids *per se*. Interestingly, the most recent lipid classification includes the above-mentioned compound groups categorized as lipids (saccharolipids and polyketides) (Liebisch et al. 2020). To understand and adjust the extraction parameters and used techniques it is important to understand the possible sources and possible lipid types that could be present in the material, in this case, berries.

A berry can be divided in 3 basic compartments – seeds, skin and pulp. In general, seeds of different berries consist of lipids that are used for energy storage, fatty acids, glycerolipids and other minor lipids. Skin consists of sterols (plant hormones, regulation of development and growth, transmembrane regulation, adaptogens) and prenol lipids (ensure various functions within the cells, act as defensive compounds against pathogens) as well as waxes (discussed in detail in Chapter 3.3. of thesis) (a mixture of long-chain aliphatic hydrocarbons, alkanes, esters, long-chain fatty acids, ketones, aldehydes, primary and secondary alcohols that protect the plants from biotic and abiotic stress) (Bederska-Łojewsk et al. 2021). Pulp contains variety of fatty acids (various length, up to C22), sterols, triterpenoids and other minor lipids, high concentration of carbohydrates and polyphenolics (Tundis et al. 2021). Considering the possible compound groups in berries and their parts, extraction solvents and techniques should be selected accordingly. Since the compounds have different polarities, a variety of extraction solvents have been tested to optimize the lipid extraction based on highest yield.

Table 3.1. Lipid extraction yield from various fresh berries using Bligh-Dyer extraction method.

Berries	Extraction yield, g extract/100g fresh berries
Bilberries	0.36
Lingonberries	0.57
Cloudberries	0.27
Black crowberries	0.46
Bog cranberries	0.37
Rowanberries	0.48
Blueberries	0.23

Bligh-Dyer extraction (Bligh and Dyer 1959) has been used for the extraction of lipids, this extraction uses a two solvent system where aqueous methanol together with chloroform has been used in the ratio of 2:1. This allows for dehydration of the fresh berry mass and simultaneous extraction of lipids. The hydrophilic compounds are also partly extracted and transferred to the aqueous methanol phase, thus giving lower total extraction yields (Table 3.1.). The other type of extraction tested was solvent extraction where pure solvents (Table 3.2.) were used – in this case berries must be dried beforehand, in order to avoid introduction of water into the organic phase. The highest yields have been obtained using the solvent extraction with the ultrasound assisted extraction technique (20 minute treatment, optimized in unpublished data) as the method of choice. As determined in **Article 1** the most suitable extraction solvent, providing the highest extraction yields, was chloroform – these results have thus been also used further for the investigation of lipids in berries (**Article 2 and 3**).

Table 3.2. Lipid extraction yield from dried blueberries depending on the used extraction solvent.

Solvent	Extraction yield, g extract/100g fresh berries
Hexane	4.62
Chloroform	7.58
Petroleum ether	4.01
Ethyl acetate	5.25
Diethyl ether	4.46
Dichloromethane	4.98

Vaccinium berries have been found to contain from 6.90 g to 9.17 g of lipids/100 g of dried berries (Table 3.3.). However, these results largely depend on the extraction method used and the parameters applied, for example, solvent: berry mass ratio, frequency and power of the ultrasound used for the extraction, total volume of extract to be prepared and others. Further research (**Article 3**) indicates that larger amount of berry biomass and larger volume of solvent are needed to extract proportional amount of berry lipids. Another aspect, regarding the different yields presented in the different studies (**Articles 1,2,3**) is the quality of the berries, in particular, the harvesting time, ripeness of the berries, sample preparation (drying of the berries, milling).

Solvent extractions, in general, have been found to provide higher extraction yields, than the Bligh-Dyer extraction method, however, the composition of the extracts must be evaluated to make a definitive conclusion on the effectiveness of the extraction. Since Bligh-Dyer method employs a 2-phase extraction approach, where aqueous methanol is used, it is evident, that groups of substances that favor more polar medium are transferred into the methanol phase, rather than the organic, chloroform phase. These substances include carbohydrates, which in fact, are not of interest in the regard to lipids. The extracts obtained by Bligh-Dyer extraction method could therefore be considered to contain "purer" lipid fraction, since the yields are much lower and the extracts don't contain any carbohydrates. However, considering the variety of lipids found in berries

Table 3.3. Lipid extraction yield from various berries using chloroform as extraction solvent.

Berries	Extraction yield, g extract/100g fresh berries
American cranberry	9.17
Bilberry	8.37
Lingonberry	9.05
Bog cranberry	7.57
Bog bilberry	8.66
Blueberry cv. 'Blue crop'	6.90
Blueberry cv. 'Blue gold'	7.84
Blueberry cv. 'Blue ray'	7.10
Blueberry cv. 'Chippewa'	7.51
Blueberry cv. 'Duke'	8.15
Blueberry cv. 'North blue'	8.18
Blueberry cv. 'Patriot'	7.65
Blueberry cv. 'Polaris'	7.46

and the wide range of polarities these compounds possess, these results should be carefully considered – methanol is considered a polar solvent, but nevertheless some lipids can also dissolve in methanol. Lipids of the fatty acid and sterol groups can also be transferred to the methanol phase, thus lowering the lipid contents in the organic phase. Considering this, the extraction of lipids for analytical study of lipid composition is suggested to be done using pure organic solvents. The appearance of typical berry extracts obtained using solvent extraction can be seen in Figure 3.5.

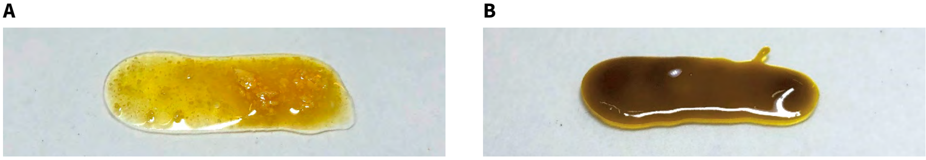


Figure 3.5. Examples of lingonberry (A) and bilberry (B) press residue lipid extracts obtained using chloroform.

Combining the solvent extraction with another type of treatment can increase the extraction yields significantly. Among the tested methods (maceration, heating with stirring) ultrasound assisted extraction has been proven to provide the highest yields at constant extraction parameters. The effectiveness of the ultrasound treatment can be attributed to the mode of action of the ultrasound – the high frequency creates micro-bubbles that after several cycles of ultrasound fluctuations implode thus creating a cavitation effect, this effect allows for penetration of the solvent into the disrupted cells and thus increases the amount of substances that are available for dissolution into the solvent (Dzah et al. 2020). Moreover, the energy delivered to the sample slightly warms the sample (not more than 40 °C) increasing the solubility of the lipid compounds. The extraction effectiveness reached a plateau after 15-20 minutes of treatment as determined during the method optimization process. Ultrasound assisted extraction was determined to be the method of choice for the solvent extraction of berry lipids.

As an environmentally friendly extraction approach with potential uses in industry, extraction with supercritical CO_2 (SCO_2) was tested and evaluated based on extraction yield (Table 3.4.). Whole, dried berry and berry press residue powders were extracted using ethanol as a co-solvent (to ensure fluidity of the obtained extract through the system). The obtained extracts have similar composition as those extracted using non-polar solvents (chloroform, hexane, petroleum ether, ethyl acetate) giving comparable extraction yields. Compared to the solvent extractions, where the solvent can be retrieved and re-used for further extractions, SCO_2 extraction re-circulates the CO_2 through the system and does not produce it during the runs and extraction procedure, also the CO_2 evaporates from the extract leaving no harmful residues in the extracts, whereas solvents can leave residues that have been introduced during the manufacturing process or added as solvent stabilizers. Considering the advantages, the development and increasing availability of SCO_2 extraction units, also on industrial scale, this approach has become a viable option for extraction of lipid substances from a variety of materials – to show the efficiency, low carbon footprint and reduced product costs through this type of extraction.

Table 3.4. Lipid extraction yield from *Vaccinium* berries using supercritical carbon dioxide as extraction solvent.

Berries	Type of material	Extraction yield, g/100g berries
American cranberries	Whole berries	8.71
	Press residues	14.48
Bilberries	Whole berries	8.22
	Press residues	10.53
Blueberries	Whole berries	7.18
	Press residues	10.45
Lingonberries	Whole berries	8.87
	Press residues	11.48

3.2.2. Qualitative and quantitative analysis of obtained lipid extracts

The obtained lipid extracts were analysed using GC/MS for their qualitative and quantitative composition. The main aim of qualitative and quantitative analysis was to identify substance present in the different berry lipid extracts based on the used material (whole, fresh berries, berry press residues) and thus support development of new applications for bioeconomy.

As the first step, solvent influence on the composition and extraction efficiency of individual substances was evaluated (**Article 1**). Five solvents were tested for their efficiency in extraction of individual lipid compounds – hexane, petroleum ether, diethyl ether, ethyl acetate and chloroform. 22 individual substances, that were present in all the prepared extracts were quantified and compared based on the solvent used (Table 3.5.). The total extraction yield of the quantified substances was the highest when diethyl ether was used, while the least effective was petroleum ether. Depending on the substance of interest, the extraction conditions should be optimized not only on the total yield of extraction (dry matter/g of biomass), but also on the specific substance of interest – this is a solution for controlling the final extract composition and obtain extracts with more specific activities, which is based on the composition.

Table 3.5. Chemical composition (μg/g of dried berries) of blueberry extracts depending on the used extraction solvent. Values represent the means (*n*=3). ND – substances not determined or lower than the LOD.

Substance	Hexane	Petroleum ether	Diethyl ether	Ethyl acetate	Chloroform
Benzoic acid	66.0	22.8	64.7	16.9	49.0
Nonanoic acid	2.75	1.92	2.77	2.65	2.65
Butanedioic acid	ND	ND	402	ND	ND
Dodecanoic acid	2.80	1.78	4.32	1.92	3.35
Citric acid	ND	ND	77.7	ND	ND
Glucofuranoside	13.9	2.10	10.5	3.18	9.1
Palmitic acid	82.2	16.1	121	1.83	119
9,12-Octadecadienoic acid	75.15	5.59	320.75	718.16	223.80
9,12,15-Octadecatrienoic acid	298	10.9	819	105.18	37.24
trans-11-Octadecenoic acid	53.61	2.58	102	36.7	65.5
Octadecanoic acid	12.8	2.58	41.7	2.87	2.70
Butyl 9,12-octadecadienoate	87.4	27.1	97.5	309.95	90.98
Butyl 9,12,15-octadecatrienoate	261.20	65.93	297	38.4	323
Butyl octadecanoate	34.5	11.5	39.2	10.3	30.9
Heptacosane	13.7	8.8	8.3	1.73	6.8
Nonacosane	15.1	2.01	15.1	3.21	15.2
Octacosanal	62.53	18.2	77.6	2.42	77.7
Triacontanal	31.2	3.14	27.8	1.86	36.6
β-Sitosterol	341	132	334	52.1	321
β-Amyrin	59.8	14.5	58.0	13.5	57.9
α-Amyrin	16.4	7.13	15.0	26.9	18.2
Betulin	8.58	3.84	15.51	17.91	24.63
Total	1,539	361	2,951	1,368	1,516

Qualitative identification of substances in the analysed *Vaccinium* berries range from 33.46 to 83.82% of all the monitored signals. The unidentified substances in the lipid extracts are believed to be complex substances with large molecular mass, for example, triterpenes bound with fatty acids, triglycerides, and others. Since limitations exist with the used method for identification and quantification, the obtained extracts could be fractionated using solid phase extraction with different sorbents – some of the substances found in the extracts are in low concentrations, and by chromatographic analysis these signals merge with the baseline and thus appear as noise. In the analysis of total extracts, a total of up to 120 substances were identified in bog bilberries, bog cranberries, lingonberries, bilberries, American cranberries and different varieties of highbush blueberries, as well as berries from other species than *Vaccinium*, crowberries, cloudberries, rowanberries. The identified compounds of berry lipids were divided in 6 lipid subclasses. The lipid subclass represented in highest concentration are the fatty acids (up to 60% of total lipids in varieties of blueberries). The main fatty acids found in the studied berries are palmitic, linoleic and linoleaidic acids (Figure 3.6).

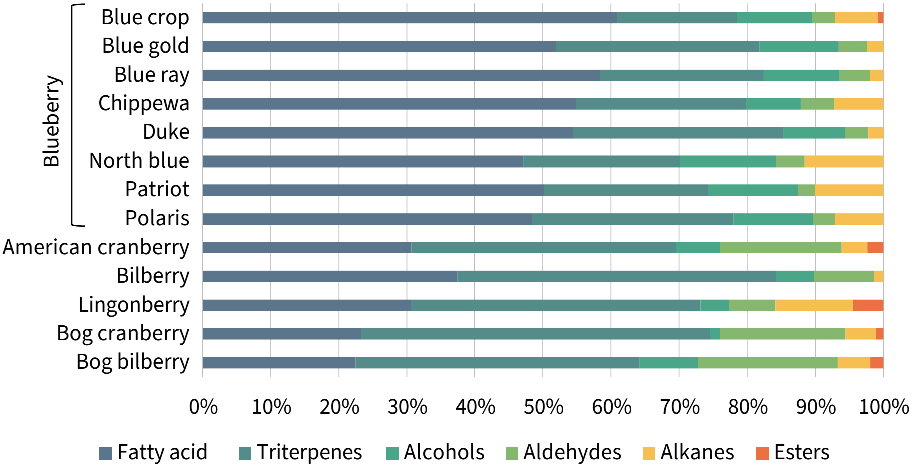


Figure 3.6. Relative abundance of identified lipid compound groups in the studied *Vaccinium* berries.

A group of substances often found as a part of lipid extracts are alkanes. These compounds are a part of berry epicuticular wax and in cytoplasm of berry they are precursors for biosynthesis of other plant metabolites. The carbon chain length of alkanes found in berries ranges from C19 to C31. As the most prominent of the alkanes in berries the C29 alkane (nonacosane) was found. Among the studied wild berries, the highest concentration of nonacosane was found in lingonberry (6.81 g/100 g). The wild bilberries and bog bilberries had low alkane concentrations, and in bilberry, only C25 and C29 alkanes were found. The studied blueberry varieties showed a much wider alkane content, for example, blueberry variety 'North blue' contained 8 different alkanes, with the most prominent alkane being nonacosane (Table 3.6.).

Table 3.6. Concentration and chain length of alkanes in the studied *Vaccinium* species berries. Values represent g of specific alkane/100 g of extract. <LOD – concentration lower than the limit of detection (0.2 μg/g).

	C19	C23	C25	C26	C28	C29	C30	C31
Bog bilberry	<LOD	0.13	0.38	<LOD	3.31	0.90	<LOD	<LOD
Bog cranberry	<LOD	<LOD	<LOD	<LOD	1.53	1.49	<LOD	<LOD
Lingonberry	<LOD	<LOD	0.13	<LOD	0.11	6.81	0.42	1.67
Bilberry	<LOD	<LOD	0.21	<LOD	<LOD	0.34	<LOD	<LOD
American cranberry	<LOD	<LOD	<LOD	<LOD	<LOD	2.33	<LOD	<LOD
‘Polaris’	<LOD	<LOD	0.22	<LOD	0.25	4.58	<LOD	0.73
‘Patriot’	0.33	<LOD	0.36	<LOD	0.45	8.01	<LOD	2.70
‘North blue’	0.28	0.24	0.33	0.25	0.66	9.33	0.56	2.80
‘Duke’	0.22	<LOD	0.20	0.20	<LOD	0.97	<LOD	0.22
‘Chippewa’	0.19	<LOD	0.62	0.44	0.38	6.05	0.29	0.91
‘Blue ray’	0.30	<LOD	0.27	<LOD	0.34	1.31	<LOD	<LOD
‘Blue gold’	0.29	<LOD	0.22	<LOD	0.15	1.03	<LOD	0.24
‘Blue crop’	0.24	<LOD	0.24	0.21	0.25	3.18	<LOD	0.89

Triterpenes and sterols serve a protective function as well as participate in a variety of cellular functions (Thimmappa et al. 2014; Valitova et al. 2016). Wild berries have been found to have higher amounts of triterpenes present, while sterols were found to be in higher concentrations in cultivated berries. In analysed berries triterpenes were found to compose 50% of identified lipids in bog cranberry and as low as 17% of lipids in blueberry varieties. The main triterpenoids in analysed berries were ursolic acid, α-amyrin, β-amyrin and sterols β-sitosterol and lupeol. Additionally, also other triterpenoids and sterols in minor concentrations have been found (Table 3.7.).

The obtained total lipid extracts of berries contain a large number of different substances from different groups of lipids and thus can have various biological effects. To evaluate and identify the groups of compounds responsible for specific activities, total lipid extraction was performed. Obtained bilberry and lingonberry extracts were fractionated using different solvents or their mixtures as eluents on silicagel column. Hexane, hexane/chloroform (1:1), chloroform, ethyl acetate, ethyl acetate/methanol (1:1) and methanol were used as eluents. The chloroform extracts were loaded onto the column and eluted with the abovementioned solvents. The least polar solvents hexane and chloroform eluted most of the loaded extract, while the more polar ethyl acetate and methanol eluted the remaining substances. The total recovery of the fractionation was 97.7 and 93.1% for bilberry and lingonberry, respectively. The obtained fractions were then analysed using GC/MS. The results indicate that some of the obtained fractions contained specific groups of compounds, while some groups of compounds were present in all the fractions (phenolic acids in lingonberries, fatty acids in bilberries) (**Article 3**).

Table 3.7. Concentration of triterpenoids and sterols in the studied *Vaccinium* species berries. Values represent g of specific sterol or triterpenoid/100 g of extract. <LOD – concentration lower than the limit of detection (0.2 µg/g).

	Ursolic acid	beta-sitosterol	beta-Amyrin	alpha-Amyrin	Lupeol
Bog bilberry	27.06	6.35	0.48	0.48	<LOD
Bog cranberry	28.23	4.61	<LOD	0.78	<LOD
Lingonberry	24.4	6.03	1.76	1.4	<LOD
Bilberry	12.75	4.56	1.52	<LOD	<LOD
American cranberry	17.08	5.56	0.92	<LOD	<LOD
‘Polaris’	12.37	8.66	2.68	<LOD	<LOD
‘Patriot’	17.18	10.72	<LOD	<LOD	<LOD
‘North blue’	10.73	11.3	3.4	0.76	2.01
‘Duke’	12.39	9.77	2.46	<LOD	<LOD
‘Chippewa’	8.75	10.97	10.66	<LOD	<LOD
‘Blue ray’	12.87	9.96	1.34	<LOD	2.07
‘Blue gold’	10.36	9.88	0.94	1.84	<LOD
‘Blue crop’	4.47	9.49	<LOD	<LOD	<LOD

Comprehensive analysis of total lipid extracts is a crucial step in identification of lipid extract application areas. Moreover, the fractionation allows for a clearer view on the fields of application, since the substances or their groups responsible for specific activities can be identified more easily – fractionation allows preparation of purer functional fractions, that will have potentially higher applicable activities. Detailed chemical analysis of the prepared lipid extracts from berries of *Vaccinium* and other species, as well as their varieties, can be found in **Articles 1, 2 and 3**.

3.2.3. Identification of lipid extract applications

Sun protection factor of berry lipids

Berry lipids possess skin moisturizing and protective abilities, which are attributed to the presence of unsaturated fatty acids (Ispiryan et al. 2021). The compositional analysis have revealed the presence of a variety of compound groups which can have other possible activities and therefore it is important to evaluate lipid fraction uses as components for cosmetic products with specific applications. Various plant extracts have high sun protection factor (SPF) and have been used as UV filters in sunscreens, however, berry lipids have not been evaluated in this regard (Sutar et al. 2020).

The obtained SPF values for total extracts were 3.6 and 9.4 for bilberry and lingonberry, respectively. Total extract of lingonberry contained high levels of phenolic acids and isoprenoids, which were absent in bilberry extract. In general, the SPF values of tested fractions increase as the polarity of the used elution solvent increases and reaches the peak SPF values when ethyl acetate/methanol was used as eluent. The highest SPF values obtained were of ethyl acetate/methanol fraction, 11.6 for bilberry and 10.2 for lingonberry (Table 3.8.). Bilberry ethyl acetate/methanol fraction consist of large amounts of fatty acids, namely the C18 unsaturated fatty acids (157 mg/g extract) and cinnamic acid (27 mg/g), while lingonberry fraction contain 27 mg/g and 11 mg/g of the respective compounds, both of the berry fractions also have high contents of benzoic acid (36 and 197 mg/g). Benzoic acid, due to its high concentration in the total lingonberry extract, is found in all of the studied fractions (up to 397 mg/g in chloroform fraction), while cinnamic acid was found only in the ethyl acetate/methanol fraction of both, lingonberry and bilberry, which indicates the UV absorptive potential of this phenolic acid. Indeed, cinnamic acid has been previously shown to have strong UV absorption capacity and it is used commercially in sunscreen products (Gunia-Krzyzak et al. 2018). Also, isoprenoids have been attributed to possess UV absorptive ability (Ramachandran and Prasad 2008), however, this cannot be confirmed in this study, as the MeOH fraction of lingonberry contained 45 mg/g oleanolic acid and 208 mg/g ursolic acid, as well as the same fatty acids and benzoic acid as the ethyl acetate/methanol fraction and showed much lower SPF values than the fractions containing cinnamic acid (Table 3.8).

Table 3.8. Determined sun protection factor values (SPF) of bilberry and lingonberry extracts and their fractions. Data represent the mean value, *n*=5. Different letters next to the analysed fraction represent significant differences according to ANOVA post-hoc Tukey's HSD, $p<0.05$.

		SPF		
Berry	Analysed fraction	1.0 mg/mL	2.5 mg/mL	5 mg/mL
Bilberry	Hexane^{b}	3.0	7.0	14.4
	Hexane/chloroform^{a}	1.3	3.1	6.2
	Chloroform^{b}	3.4	8.3	15.5
	Ethyl acetate^{d}	7.2	16.6	33.7
	Ethyl acetate/methanol^{c}	11.6	25.5	53.0
	Methanol^{c}	6.8	16.3	32.5
	Total extract^{b}	3.6	9.0	18.7
Lingoberry	Hexane^{a}	2.6	5.8	12.3
	Hexane/chloroform^{b}	4.5	10.4	18.9
	Chloroform^{c}	8.2	19.9	40.2
	Ethyl acetate^{c}	7.8	19.1	36.7
	Ethyl acetate/methanol^{c}	10.2	24.0	49.0
	Methanol^{b}	3.9	10.1	17.9
	Total extract^{c}	9.4	21.6	44.0
	Control (chloroform)	0.0	0.0	0.0

The SPF values obtained show that the use of berry lipids have a potential application in sunscreen production, thus substituting synthetic and inorganic sunscreen constituents to natural and sustainable components with low environmental impact, at the same time promoting bioeconomy and valorisation of food wastes.

Antimicrobial activity of berry lipid fractions

The antimicrobial activity of total berry lipid extracts and extract fractions was tested against 6 human pathogens or opportunistic pathogens *via* agar-well diffusion method. As a result, the obtained fractions exhibited antimicrobial activity against *S.aureus, S.pyogenes, S.epidermidis* and *E.coli*, but no activity against *P.mirabilis* and *P.aeruginosa* was detected. Bilberry total extract and its fractions demonstrated high inhibition potential when tested with *S.aureus*. Chloroform and ethyl acetate fraction demonstrated significant antimicrobial activity, these fractions were especially rich in β-sitosterol, containing 280 mg/g and 70 mg/g of this substance, respectively. These fractions also showed inhibitory effect against the other Gram-positive bacteria, *S.pyogenes* and *S.epidermidis*. *S.pyogenes* was inhibited by all of the tested bilberry fractions, except the most non-polar, hexane fraction, where the main compound groups were aldehydes with nonacosanal (111 mg/g) and fatty acids with oleic acid (119 mg/g) as the main constituents. The only Gram-negative bacterium inhibited by the tested berry lipid fractions was *E. coli*. Bilberry ethyl acetate/methanol and methanol fraction showed significant inhibition and were particularly rich in phenolic acids (141 mg/g of quininic acid in methanolic fraction) and fatty acids (249 mg/g malic acid in methanolic fraction). The inhibition presented by the total lipid extract is similar to the prepared fractions, which indicates there is possible synergistic effect of the berry lipids which reduces microbial growth.

The tested lingonberry extract fractions and total extract contained high levels of benzoic acid. Similarly to the bilberry fractions, where the Gram-negative *E.coli* was inhibited possibly due to the fatty acid contents, also lingonberry extracts showed inhibition of this bacterium. The lingonberry methanol fraction contained isoprenoids oleanolic acid and ursolic acid, however, these fractions did not show higher inhibition potential. Interestingly, the hexane and hexane/chloroform fractions inhibited the growth of *S.pyogenes* – these fractions had high levels of squalene (185 mg/g), β–sitosterol (26 mg/g) and lanosterol (24 mg/g).

The broad spectrum of compound groups found in the berry lipids make them attractive as antibacterial agents for various applications in food preservation, medicine, as part of nutraceuticals, cosmetics and could possibly reduce the use of conventional antibiotics. Depending on the microorganisms that have to be controlled, berry lipids could be added to various products to control or minimize the possibility of food spoilage and foodborne pathogens. Cosmetic products containing these antimicrobial and sun protective berry lipids are a safe alternative to the conventionally used cosmetic products – it could possibly help in avoiding allergens and the development of antibiotic resistant skin pathogen strains. Results obtained in this study can help in elucidation of possibly new fields of application of lipid compounds. The demand for safe, natural food and cosmetic ingredients increase as the overall population becomes more involved and aware of the health and environmental issues proposed by the modern industrial solutions. Berry lipids are a largely neglected group of compounds, however, from the point of application potential they are one of the most valuable groups of compounds supporting health promoting value of berries. The research on the composition and use of natural products becomes more important, as environmentally friendly alternatives of various conventional ingredients must be found. As the food industry and berry processing steadily increases worldwide, berry lipids, which could be extracted from berry press-residues, can play a significant role in new, user friendly, innovative product development.

3.3. Berry and their press residue wax

Berry and plant waxes in general consist of a mixture of long-chain aliphatic hydrocarbons, alkanes, esters, ethers, long-chain fatty acids, ketones, aldehydes, primary and secondary alcohols that protect the plants from biotic and abiotic stress. The aim of the study on berry and their press residue wax was to understand the functions of berry cuticular wax as part of the plant's defence system, to demonstrate possibilities of berry wax practical application, considering wax as one of the functional components derived from the biorefinery process (Figure 3.7).

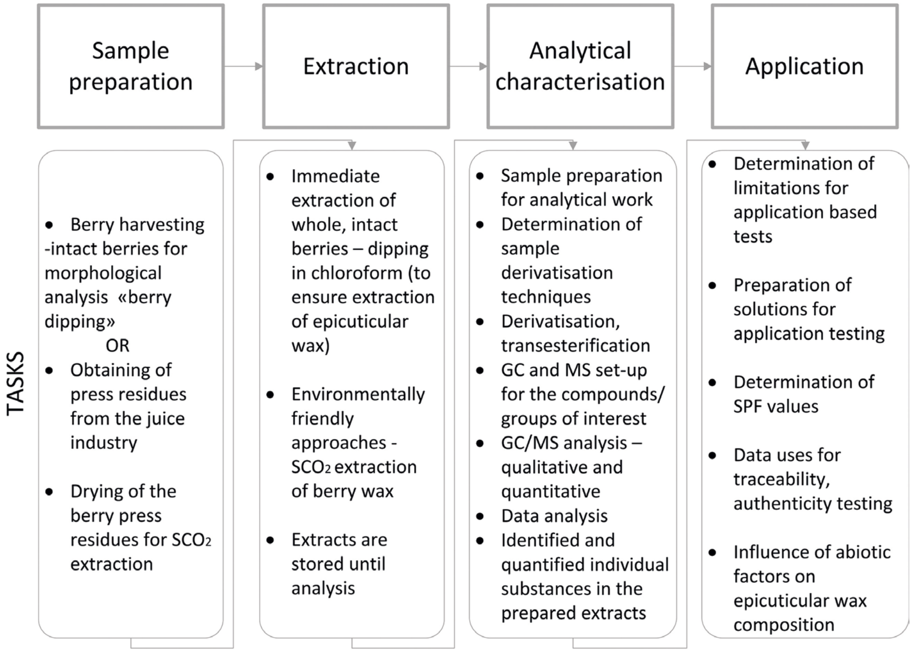


Figure 3.7. Berry biomass wax extraction and research flowchart.

3.3.1. Extraction of berry wax

In the thesis the main work has been done on cuticular wax layer of berries, which consists of intracuticular wax (amorphous lipids embedded in cutin) and outermost epicuticular wax (Jetter et al. 2008). Since it is not possible to divide the two layers of wax present on the outer part of the berry, both of these layers must be extracted simultaneously. To isolate the berry cuticular wax layer specifically, fresh berries must be used. Berry press residues contain a mixture of berry parts, therefore the extraction and determination of cuticular wax composition of such material is not possible and therefore the compositional analysis of cuticular wax must be done using intact berries. However, berry press residue wax can be obtained as a product using SCO_2 extraction, which gives a possibility to prepare berry waxes on semi-industrial scale.

The morphology of berry wax differs depending on the crystal forming substances that are part of the wax. Generally, two distinct phenotypical morphological groups of waxes on the surface of plant organs can be distinguished – glaucous (bilberries, bog bilberries, blueberries) and glossy (lingonberries, bog and American cranberries, crowberries). However, despite the apparent similar appearance of, for example, blueberries, bilberries, bog bilberries, that can be seen with the naked eye, scanning electron microscopy (SEM) reveals different morphological wax structures on these berries. Classification of plant wax morphology has been elaborated and specific structural and chemical diversity can be determined by SEM analysis (Barthlott et al. 1998; Jeffree 2006). The chemical composition affects the morphological characteristics of the berry surfaces thus giving the berries their specific appearance. The performed SEM analysis together with wax extraction yields show that the berries with glaucous appearance have higher wax density than the glossy berries. The amount of wax on bilberry and bog bilberry was 871.1 and 921.8 $\mu g/cm^2$ while on lingonberry and crowberry it was 331.3 and 108.5 $\mu g/cm^2$ (**Article 4**).

Extraction of cuticular wax of different berry species was done using chloroform as suggested earlier (Jetter et al. 2008). Examined berries were bog bilberry (*Vaccinium uliginosum* L.), bilberry (*Vaccinium myrtillus* L.), American cranberry (*Vaccinium macrocarpon*), lingonberry (*Vaccinium vitis-idaea* L.), black crowberry (*Empetrum nigrum* L.), gaultheria (*Gaultheria mucronata*), rowanberry (*Sorbus aucuparia* L.), hawthorn (*Crataegus alemanniensis*) and eight varieties of blueberry (*Vaccinium corymbosum* L.), namely, 'Blue crop', 'Blue gold', 'Chandler', 'Chippewa', 'Duke', 'North blue', 'Patriot' and 'Polaris'. Fresh, intact berries were submerged into fresh chloroform and then, to ensure complete washing-off of the cuticular wax layer, they were dipped 2 times more in separate flasks with pure chloroform. Such approach ensured, that only the outermost layer of the berries was subjected to extraction, thus avoiding leaching of cytoplasmic lipids into the extract. The obtained extraction yields (Table 3.9.) suggest that the berries that have glossy, smooth cuticular wax layer (not forming wax crystals), like lingonberry, crowberry, rowanberry and cranberries have higher cuticular wax concentration than the berries that have white, textured cuticular wax layers (crystal forming), like blueberries and bilberries. The extraction methodology was kept constant throughout the series of wax investigation.

Table 3.9. Studied berries, their taxonomic relation based on family and species and the amount of wax in mg per berry. ± represents the standard deviation of the wax amount (*n*=3).

Studied berry	Family	Species	Variety	Wax, mg/berry
Hawthorn	Rosaceae	*Crataegus*		1.43 ± 0.09
Rowanberry	Rosaceae	*Sorbus*		1.48 ± 0.09
Gaultheria	Ericaceae	*Gaultheria*		0.65 ± 0.02
Black Crowberry	Ericaceae	*Empetrum*		1.71 ± 0.11
Bog bilberry	Ericaceae	*Vaccinium*		0.95 ± 0.09
Bilberry	Ericaceae	*Vaccinium*		0.63 ± 0.05
Lingonberry	Ericaceae	*Vaccinium*		1.89 ±0.09
American cranberry	Ericaceae	*Vaccinium*		1.46 ± 0.12
Blueberry	Ericaceae	*Vaccinium*	'Blue crop'	0.74 ± 0.04
			'Blue gold'	0.67 ± 0.03
			'Chandler'	0.83 ± 0.05
			'Chippewa'	0.90 ± 0.07
			'Duke'	0.57 ± 0.02
			'North blue'	0.65 ± 0.02
			'Patriot'	0.84 ± 0.03
			'Polaris'	0.87 ± 0.03

The wax amount of bilberries was investigated throughout the fruit development for wild-type bilberry and glossy-type (natural bilberry mutant) to investigate the wax layer development as the berry ripens. It was found that during the ripening stages the amount of wax on the berry surface slightly increased for both types of berries reaching, though, no significant differences between the wild-type and glossy-type berries were detected in any of the investigated ripening stages. The wax amount per area of berry surface also showed no significant differences among wild-type and glossy-type, the wax density slightly increased at the later development stages, although not significantly (**Article 6**).

The latitude at which the berries have been harvested is believed to have influence on the chemical composition of the berries, including the composition and amount of wax on the berry surface. This hypothesis was tested by gathering bilberries and extracting their cuticular wax from Latvia, Norway and Finland in two consecutive summer seasons (year 2018 and 2019). The results firstly showed that depending on the harvest year, the wax load (mg wax/berry) can change significantly and secondly, the amount of wax load changes depending on the latitude at which the bilberries have been harvested. The wax is largely responsible for the protective functions of plants, and thus it can change depending on the abiotic environmental factors. Correlation analysis show that the wax load has a statistically significant negative relation with meteorological data (precipitation) two months pre-harvest – the influence of environmental parameters on bilberry wax is further explored in Section 3.3.3 of the thesis and in **Article 7**.

SCO_2 extraction was done using dried, milled berry press residues and waxy, dry material was obtained in preparative amounts. Since berry press residues contains other berry compartments, including seeds, the extraction yields obtained have been higher and also the wax appearance was different, due to the lack of selectivity while using such an extraction approach. The extracts prepared using SCO_2 extraction had yields of 1.02% and 0.45% for lingonberry and bilberry press residues, respectively, while berry dipping in chloroform, where the cuticular wax layer is extracted specifically, results ranging from 0.05-0.1%, depending on the berries, were achieved. However, the extracts prepared using SCO_2 contain substances that can, in fact, improve the quality and application possibilities of the prepared wax extracts. SCO_2 extraction showed the possibility to extract wax from juice industry waste, while creating a valuable ingredient that can be used in food or cosmetic industries.

3.3.2. Qualitative and quantitative analysis of wax extracts

Berry wax constituents can be divided into nine groups of compounds – alkanes, phytosterols, triterpenoids, alcohols, fatty acids, phenolic acids, ketones, tocopherols and aldehydes. Depending on the berry and its cuticular wax morphology, the composition can differ, and the major compound groups can vary among not only species but also cultivars of the same species (Figure 3.8A). Phenolic acids and tocopherols were discovered to be minor constituents of berry wax; despite their relatively modest quantities, they play a crucial part in the interaction between plants and pathogens. According to reports, phenolic acids and tocopherols can protect against UV radiation and support antibacterial activity, respectively (**Articles 4 and 5**). Triterpenoids, fatty acids and alcohols are the three main compound classes of cuticular wax, accounting for up to 62%, 31% and 38% of the total wax content in blueberry cultivars 'Blue Gold', bilberry and 'Blue Crop', respectively. The composition and distribution of chemical compound classes can differ depending on the place of origin and genetic background (**Articles 6 and 7**).

Triterpenoids were found to be the most abundant cuticular wax constituents in the studied berry species varying from 32 to 68% of the total amount of wax compounds. In total eleven different triterpenoids were identified in varying amounts. Ursolic acid was the most abundant triterpenoid, followed by α-amyrin, β-amyrin, β-sitosterol, lupeol, lanosterol and uvaol, followed by others in minor concentrations (Figure 3.9).

Alkanes with the chain length C20-C30 have been found in the cuticular wax of berries in the concentration range from 1.5 to 14% depending on the berry species and cultivar (Figure 3.8). The dominant alkanes found on the surfaces of the berries have odd-numbered chain length. The main alkanes found in the cuticular wax of the studied berries were the C29 (nonacosane) and C31 (hentriacontane) alkanes (Figure 3.9).

Saturated fatty acids contribute to 26% of total wax in bilberry and 20% in bog bilberry. Blueberry varieties 'Chippewa' and 'Chandler' contained 24% and 20% fatty acids of total wax content (Figure 3.8A). Overall, the fatty acid distribution in the studied berries was higher in the *Vaccinium* species blueberries and bilberries than in the rest of the berries. Bilberry and bog bilberry present hexacosanoic acid (C26-0) as the major fatty acid, with 7.6 and 5.2 g/ 100 g extract, respectively (Figure 3.9) (**Article 5**). The appearance of the cuticular wax is largely dependent on the composition, for

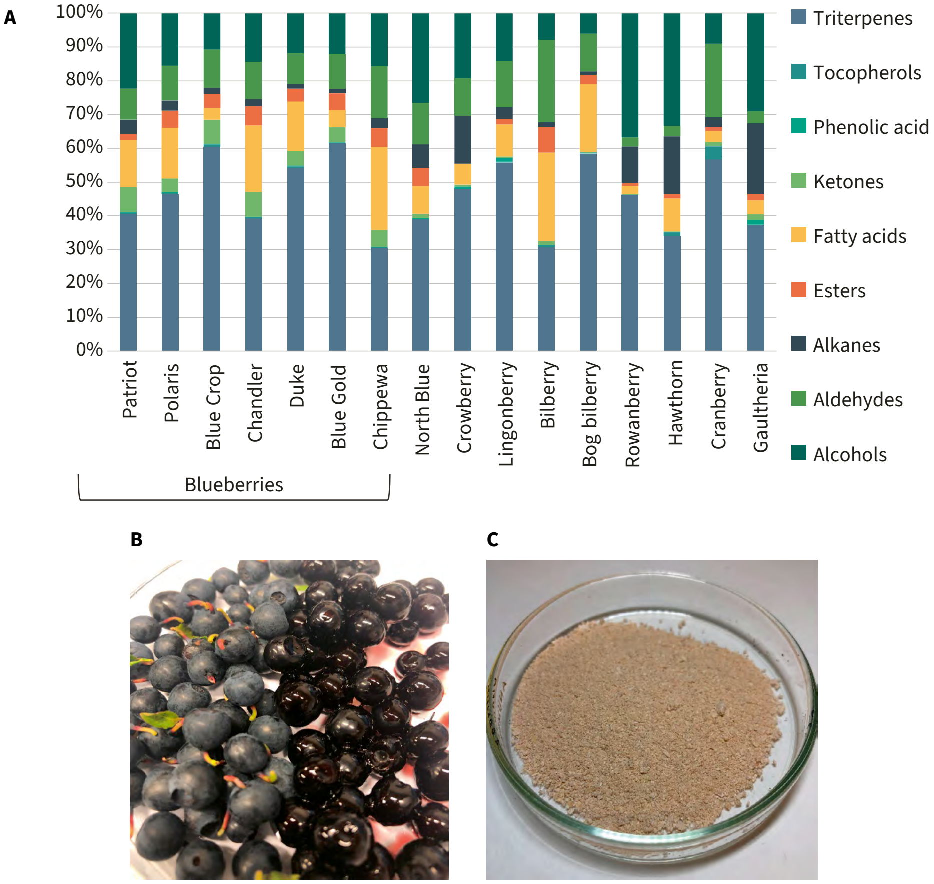


Figure 3.8. Relative amounts of identified compound classes in waxes of different berries (A); harvested bilberries on left with visible glaucous wax layer, chloroform-dipped bilberries on the right where the wax layer has been extracted (B); and bilberry cuticular wax extracted using the chloroform dipping method (C).

example, bilberry wax appears as a white, fine powder after the cuticular wax extraction (Figure 3.8B).

The cuticular wax composition of bilberries in different development stages was examined. Similarly to the results presented in **Article 5**, also the wild-type and glossy-type bilberry cuticular wax consisted mainly of triterpenoids and fatty acids as major compound classes. The glossy-type berries showed lower amounts of fatty acids and higher amounts of triterpenoids throughout the development stages as the WT berries. For both types of berries, the later ripening stages showed increase of alkanes in the cuticular wax (**Article 6**).

Triterpenoids, fatty acids, alkanes, aldehydes, ketones, and primary alcohols were the main chemical components of bilberry fruit cuticular wax that were discovered in all three locations studied in **Article 7**. Esters or secondary alcohols were not found. Through the latitudinal gradient and harvesting seasons, bilberry cuticular wax showed

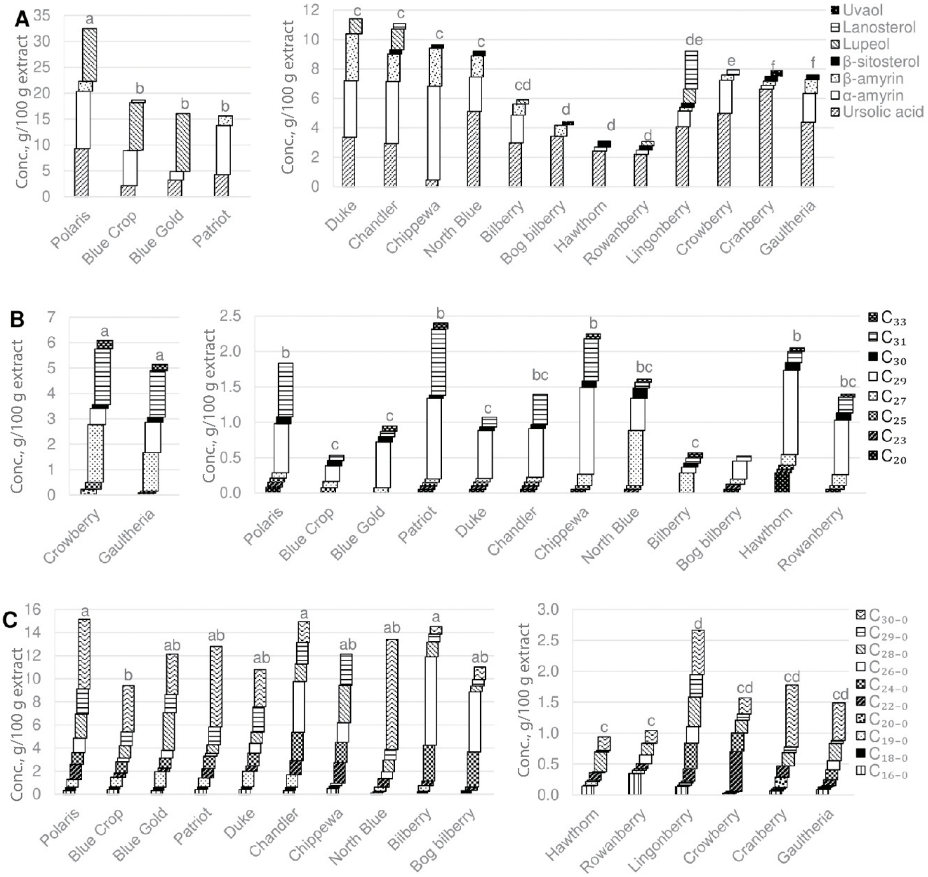


Figure 3.9. Concentration and composition of sterols (A); alkanes (B); and fatty acids (C) in studied berry waxes. C16–C33 in (B) represent the chain length of alkanes (number of C atoms). C16-0–C 30-0 in (C) represent the length of fatty acids, where $_{-0}$ represent the number of double bonds in the fatty acid molecule. Crowberry – black crowberry. Cranberry – American cranberry. Letters above the bars represent significantly different post-hoc (ANOVA, Tukey's HSD) pairwise comparison of total concentration of measured substances in respective berry.

variations in wax component concentrations and relative proportions. From southern latitudes in Latvia (79.9% triterpenoids of total wax), to locations in Finland (50.7% triterpenoids of total wax), to northern Norway (27.4% triterpenoids of total wax), a consistent trend of decreasing the proportion of triterpenoids in berry wax was seen in 2018. From southern to northern latitudes, a concurrent trend of increasing the amount of fatty acids and alkanes was seen. In the 2018 season, fatty acids predominate in northern Norway, whereas triterpenoids dominated in the bilberry fruit cuticular wax in Latvia and Finland. From the southernmost location in Latvia (42.9% triterpenoids of total wax) to the location in Finland (29.8% triterpenoids of total wax), a similar pattern of decreasing triterpenoid proportions was seen in the 2019 harvest season, along with an increase in fatty acid and alkane proportions. The same trend in triterpenoid and fatty acid proportions was not seen in the samples from northern Norway in 2019. Triterpenoids were the second-most prevalent compound in 2019 after fatty acids across all locations (**Article 7**).

SCO_2 extraction from bilberry and lingonberry press residues show the possibility to extract berry waxes by altering the extraction parameters. Since berry press residues contain seeds and skins, the extracted wax composition is significantly different than that extracted for analytical purposes using chloroform. Berry wax extracted with SCO_2 shows high contents of fatty acids – up to 83.4% and 76.9% in bilberry and lingonberry wax, respectively. As opposed to the major fatty acid extracted using chloroform, the SCO_2 extract contain high amounts of unsaturated fatty acids, rather than saturated. The major fatty acids in SCO_2 extracts were linoleic and γ-linolenic acids, constituting approximately 50% of the total wax amount. The major triterpenoids in bilberry and lingonberry press residues were found to be lupeol and β-amyrin. Alkanes, sterols, ketones, alcohols and other wax constituents were found only in minor concentrations in the SCO_2 extracts. The high fatty acid contents are associated with the presence of seeds in the press residues, which are rich in unsaturated fatty acids, the unspecific selectivity of SCO_2 allows the retrieval of these compounds (**Article 4**).

Qualitative and quantitative analysis of wax extracts and their constituents suggest that the wax composition is largely dependent on the genetic background of the berries in question, the place of origin and the extraction technique used. While extraction using chloroform (berry dipping) specifically isolates they outermost wax layer, the SCO_2 extraction is more intensive, especially for berry press residues, which is a mixture of all the berry compartments. While cuticular wax extracts isolated for use in analytical characterization contained no unsaturated fatty acids, the presence of them in SCO_2 extracts can be an advantage for incorporation in different products, especially cosmetics. Unsaturated acids are a crucial part of human diet as well, implicating possible uses of SCO_2 extracts into food articles or other innovative functional foods. As the research presented in this thesis and corresponding articles (**Article 4 and 7**) berries of different origin can have higher triterpenoid or fatty acid contents depending on the place they have been harvested. This indicates the possibility to use environmentally friendly extraction techniques for the preparation of specific functional ingredients either high in triterpenoids or fatty acids and thus the use of these ingredients in different types of products. Also, other *Vaccinium* spp. berries and their press residues could be extracted using the same method possibly allowing to obtain extracts high in other functional ingredients.

3.3.3. Identification of wax extract applications

Determination of sun protection factor (SPF) of berry wax

Sun protection factor is a value that is determined for products with the ability to block the UV-B rays which may have adverse impacts on human skin. Commonly nanosized ZnO (more effective against UV-A) or TiO_2 (more effective against UV-B) in addition to several synthetic ingredients are used as part of the commercially available sunscreens. The ability to reflect, absorb and scatter the UV-B radiation is reason why these substances are used in the sunscreens as they act as a physical barrier on the outer layer of the skin and do not allow the UV-B or UV-A radiation to come in contact with the skin. Although metal oxides like ZnO and TiO_2 were once thought to be safe physical sunscreens, it is not recommended to use them over the long term. Researchers discovered that these metal oxides release extremely reactive free radicals and reactive oxygen species when they are photoactivated by UV radiation. It was discovered that these radicals were cytotoxic and genotoxic, harming DNA and skin cells. Considering the negative effects on the environment and human health, more neutral solutions for UV protection should be considered. One such solution could be the use of berry lipids (Section 3.2.3.) or wax as a product of food waste biorefinery.

The obtained results on the berry wax and berry press residue wax show a dose dependent increase of the SPF values. Chloroform extracted berry cuticular wax of bog bilberry showed highest SPF values along the concentration gradient, reaching the SPF value of 25.42 at 2 mg/L. This SPF value is comparable to commercially available sunscreen products. Bilberry and lingonberry press residue wax extracted using SCO_2 show higher SPF values than those extracted using chloroform. The compositional analysis showed that the press residue wax extracts contain higher levels of unsaturated fatty acids, which could be the reason for higher SPF values. To investigate which substances are responsible for the UV protection ability in the wax extracts, they should be fractionated into several fractions and analyzed using GC-MS for their quantitative composition. Cinnamic acid and vitamin E, which were both present in greater amounts in bilberry wax compared to lingonberry wax (SCO_2 extractions) and significant levels of which were also discovered in bog bilberry cuticular wax, may have contributed to the high SPF (Table 3.10.). Considering the wide variety of naturally occurring compounds that potentially possess UV protective ability, the specific substances present in the berry wax should be further investigated using different types of extracts as well as different methods for the determination of UV absorption/reflection/scattering (**Article 4**).

Table 3.10. Berry wax SPF values at different wax concentrations extracted using chloroform from whole berries and press residues using SCO_2.

	1 mg/mL	2 mg/mL
Berry cuticular wax (chloroform)		
Bilberry	4.5 ± 0.9	7.5 ± 1.8
Lingonberry	4.8 ± 1.5	12.7 ± 0.8
Bog bilberry	14.8 ± 1.3	25.4 ± 0.2
Berry press residue wax (SCO_2)		
Bilberry	7.9 ± 0.3	15.09 ± 0.12
Lingonberry	6.1 ± 0.5	13.4 ± 0.9

[*] ± represents SD of the measurements (n=3).

Authenticity testing using quantitative berry wax data

Principal Components Analysis (PCA), a type of multivariate analysis, is frequently used to depict complex analytical data and make it easier to find similarities and differences across investigated clusters or groups of variables. This instrument was utilized

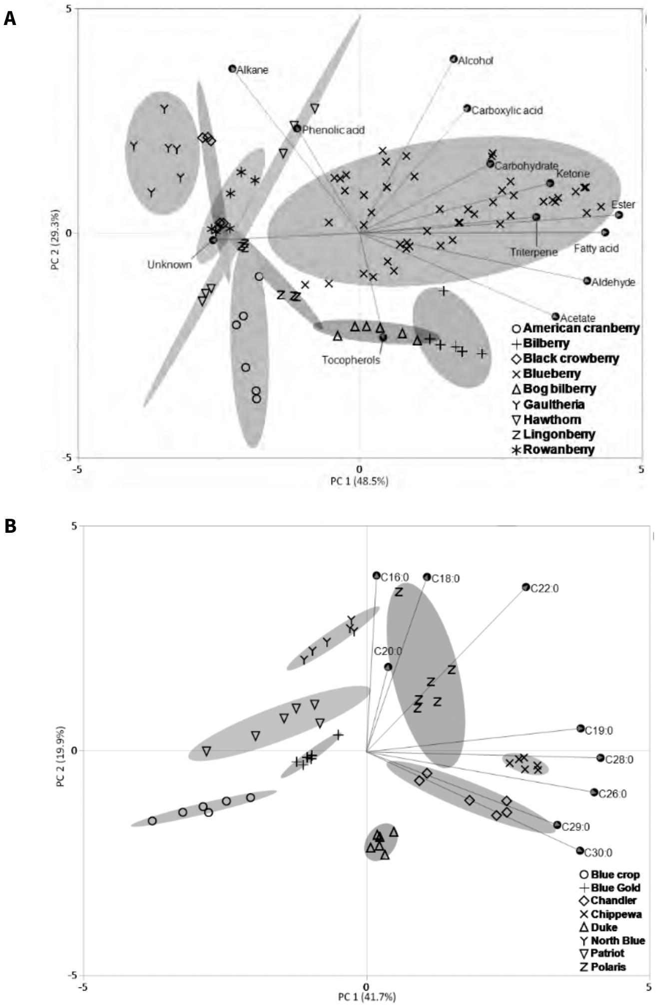


Figure 3.10. Principal components analysis (PCA) using cuticular wax quantitative analysis of tested berry species and varieties. (A) PCA scores and loadings of different compound groups found in the tested berry species. (B) Fatty acid composition in the tested blueberry varieties. Ellipses represent 95% confidence intervals.

to detect the chemicals responsible for inter- and intra-species variability and to distinguish differences in the wax compositions of the analysed berry samples. The PCA plots and loading plots demonstrate that differences in the concentration of chemicals from various groups can account for the observed differences. Figure 3.10A demonstrates the strong correlation between certain chemical groups and the clustering of blueberries, including fatty acids, triterpenoids, and esters. The clustering of berries like rowanberry, black crowberry, and gaultheria is related to the contents of unknown compounds and alkane contents, as seen on the other side of the graph. The data on blueberry varieties can be divided into discrete groups when plotted independently

(95% confidence ellipses do not overlap) (Figure 3.10A). Figure 3.10B shows that each of the analysed blueberry varieties has a unique composition of fatty acids as part of their cuticular wax, in contrast to the clustering of the various species, which shows that rowanberry, gaultheria, cranberries, and hawthorn cannot be distinguished based on their fatty acid contents. This suggests that the fatty acids and the variations in them in blueberry cuticular wax can be used as chemometric tools to distinguish between different varieties. However, these results should be carefully interpreted because the environmental factors that determine the composition of cuticular wax are highly variable from harvest to harvest.

As demonstrated in **Article 5** the compositional analysis of berry wax can be successfully used to distinguish between different berry species and even cultivars of the same species. Considering the popularity of berries and berry-based products in the market, adulteration of products becomes increasingly worrying. Northern bilberries, which are regarded as having high potential of health benefits are often substituted with cheaper blueberry, black currant or other dark-colored berry powders in products. Also, bilberry oils are added to a variety of cosmetic products, and since this is a relatively expensive ingredient, it is often substituted with other oils, that are cheaper. The use of methods demonstrated in **Article 5** shows the possibility to effectively use chemical analysis to successfully establish the authenticity of berry wax. Routine use of such methods would require creation of a chemical database, that would be regularly updated with berry samples depending on the region of origin and season of harvest (year of harvest).

Climate conditions affect berry wax composition and content

Triterpenoids, fatty acids, alkanes, aldehydes, ketones, and primary alcohols were the main chemical components that were discovered in bilberry wax sampled in Norway, Latvia and Finland (Figure 3.11), however, esters or secondary alcohols were not found. Through the latitudinal gradient and between years, bilberry cuticular wax showed variations in wax component concentrations and relative proportions. From southern latitudes in Latvia (79.9% triterpenoids of total wax), to locations in Finland (50.7% triterpenoids of total wax), to northern Norway (27.4% triterpenoids of total wax), a consistent trend of decreasing the proportion of triterpenoids in berry wax was seen in 2018. From southern to northern latitudes, a concurrent trend of increasing the amount of fatty acids and alkanes was seen. In the 2018 season, fatty acids predominate in northern Norway, whereas triterpenoids dominated in the bilberry fruit cuticular wax in Latvia and Finland. From the southernmost location in Latvia (42.9% triterpenoids of total wax) to the location in Finland (29.8% triterpenoids of total wax), a similar pattern of decreasing triterpenoid proportions was seen in the 2019 growing season, along with an increase in fatty acid and alkane proportions. The same trend in triterpenoid and fatty acid proportions, though, was not seen in the samples from northern Norway in 2019. Triterpenoids were the second-most prevalent compound in 2019 after fatty acids across all locations (**Article 7**).

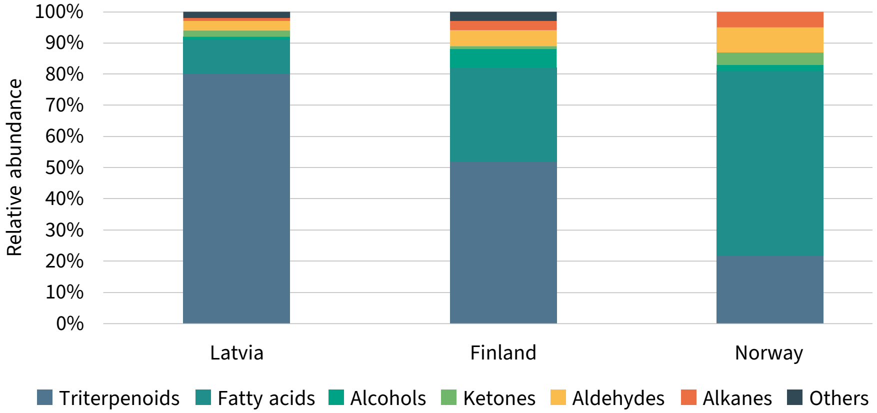


Figure 3.11. Cuticular wax profile of bilberries gathered in Norway, Finland and Latvia in the summer season of 2018.

The variance in cuticular wax composition of berries from different latitudes was examined using redundancy analysis (RDA). The research found a distinct difference between the bilberries that were gathered in 2018 from three different places. Redundancy analysis showed that the triterpenoid and fatty acid content of the cuticular wax were negatively correlated. Triterpenoid concentration showed a relation with the maximum and average temperatures in 2018. Fatty acids exhibited a negative relation with the average and maximum temperatures in 2018. In 2018, there was a substantial negative association between average and maximum temperatures and concentrations of alkanes, ketones, and aldehydes as well. According to the RDA, temperature has a favourable impact on the triterpenoid concentration of cuticular wax as well as its composition (Figure 3.12). To determine the significant environmental variables and temporal periods that may have influenced the bilberry fruit cuticular wax composition, a stepwise linear regression analysis was carried out. The investigation revealed that the climate conditions had a significant impact on the variation in triterpenoid composition (R^2 = 88%). From the start of the summer season, there was a positive correlation between the fraction of triterpenoids and both maximum and average temperature.

Observed results in the seasons of 2018 and 2019 show variability of berry wax composition, depending on geographical (location) and climatic conditions. However, to obtain decisive conclusions it could be suggested to continue sampling and analysis of cuticular wax along a latitudinal gradient to better understand the effects of weather variables on the contents and composition of berry wax (**Article 7**). Such analysis could support the possibility to produce berry derived products with specific composition based on their place of origin and thus the compositional characteristics. Moreover, the shown climatic condition and composition relations indicated plant adaptation based on the weather variability and thus would be of importance to understand climate change impacts at molecular level on cultivated and wild plants.

Studies of berry and their press residue waxes support development knowledge on plant protective mechanisms against stress, composition variability depending on geographical and climatic factors. In-depth study of berry wax composition and

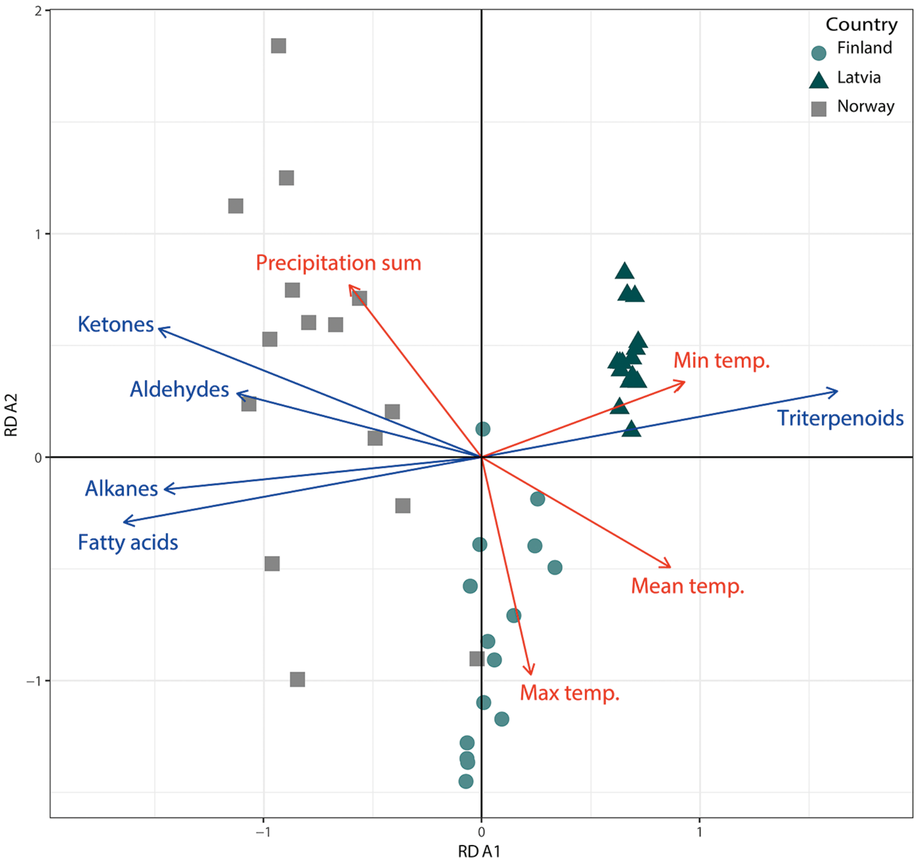


Figure 3.12. Redundancy analysis showing the relation between compounds found in berry wax and weather variables in different latitudinal locations in the summer of 2018.

demonstration of environmentally friendly extraction possibilities support application possibilities and processing of juice production wastes.

3.4. Berry and their press residue polyphenolics

Polyphenolics in berries comprise a large group of secondary metabolites. Polyphenolics are present in the berry skin and pulp and also in the seeds. The main group of polyphenolics in berries (especially the highly pigmented berries) is anthocyanins. This part of thesis concentrates on the extraction and its optimization of polyphenolics, especially anthocyanins, characterization of the extracts and possible application fields of the extraction/purification techniques and extract properties (Figure 3.13).

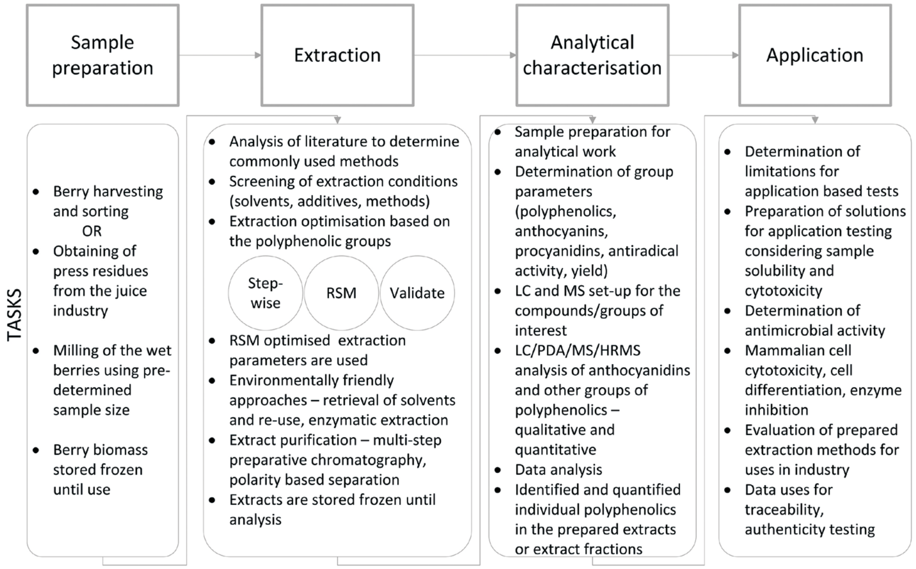


Figure 3.13. Berry biomass polyphenolic extraction research flowchart

3.4.1. Extraction optimization of berry polyphenolics

Determination of possible extraction solvent composition

Valorisation of food industry wastes (berry press residues) is a promising source of natural antioxidants – polyphenolics. The composition of polyphenols in plant material depends on plant species and their distribution in different tissues. Large amounts of polyphenols are bound in berry seeds and skin, which makes the release of these compounds difficult. The extraction conditions provided for one plant cannot be directly used for the extraction of polyphenolics from another plant due to the specific localisation of polyphenolics in various species. Therefore, an extraction method specifically for *Vaccinium* berry press residues has been optimized (**Articles 8, 9**).

Information on extraction solvents that have been used for extraction of polyphenolics from other types of material were gathered and tested on berry press residues to identify the extractant that provided highest polyphenolic and anthocyanin yields (**Articles 8, 9**). The highest extraction yields (48.38 g /100 g berries) were obtained using methanol and 1% HCl (v/v). This extraction also gave the highest amount of total anthocyanins (0.451 g /100 g berries) and polyphenols (4.8 g 100 /g berries) (Table 3.11.). However, the stability of anthocyanin molecules must be considered when using this system. The easy use and low costs of ethanol and the high polyphenolic yield obtained from the use of this solvent (3.43 g /100 g berries) support the selection of lower alcohols (ethanol, methanol) for further optimisation of extraction (solvent composition).

Table 3.11. Comparison of different solvent mixtures used for the extraction of polyphenolic compounds/anthocyanins. Uncertainty represents standard deviation. All solvents were used as v/v%. Different letters next to the values represent a significant difference in the results ($p \leq 0.05$, Student's t-test)

Extraction solvent	Dry residue, g/100 g	Carbohydrates, g/100 g	Anthocyanins, g/100 g	Polyphenolics, g/100 g
Acetonitrile 49.5%, TFA 0.5%	37.2 ± 1.5^{b}	7.8 ± 0.3^{c}	0.228 ± 0.006^{b}	3.84 ± 0.12^{b}
Acetone 50%	34.3 ± 1.4^{b}	12.2 ± 0.4^{b}	0.151 ± 0.004^{b}	2.70 ± 0.08^{c}
Acetone 75%	36.0 ± 1.5^{b}	18.5 ± 0.7^{a}	0.156 ± 0.004^{b}	2.69 ± 0.08^{c}
Methanol 60%, acetone 30%	37.4 ± 1.6^{b}	16.7 ± 0.6^{a}	0.184 ± 0.005^{b}	2.34 ± 0.07^{c}
Methanol 99%, HCl 1%	48.4 ± 2.0	17.9 ± 0.6^{a}	0.451 ± 0.011^{a}	4.80 ± 0.14^{a}
Water, HCl 1%	16.9 ± 0.7^{a}	14.8 ± 0.5^{ab}	0.098 ± 0.002^{c}	0.89 ± 0.03^{d}
Ethanol 70%, HCl 1%	39.2 ± 1.6^{b}	16.9 ± 0.5^{a}	0.204 ± 0.005^{b}	3.43 ± 0.09^{b}
Methanol 99.9%, TFA 0.1%	41.1 ± 1.6^{b}	12.5 ± 0.4^{b}	0.223 ± 0.005^{b}	2.80 ± 0.07^{c}
Ethanol 99.9%, TFA 0.1%	37.9 ± 1.5^{b}	12.1 ± 0.3^{b}	0.170 ± 0.004^{b}	1.61 ± 0.04^{c}
Methanol 95%, formic acid 5%	39.7 ± 1.6^{b}	13.1 ± 0.3^{b}	0.695 ± 0.017^{a}	4.84 ± 0.12^{a}
Methanol 99%, TFA 1%	38.8 ± 1.6^{b}	15.5 ± 0.4^{ab}	0.205 ± 0.005^{b}	3.61 ± 0.09^{b}

To increase the anthocyanin stability in the final extract and the overall extractability of polyphenolics and anthocyanins, the extraction solvent is usually supplemented with acid, to lower the pH and thus increase the solubility and stability of anthocyanins. To identify the most optimal acid for the extraction of anthocyanin and phenolics, a series of ultrasound-assisted extractions were performed, where the solvent (96% ethanol) was mixed with various acids (at 1%, v/v). Obtained results indicate that the addition of HCl significantly increased the amount of extracted polyphenolics (non-anthocyanin polyphenolics) while TFA supported extraction of anthocyanins more effectively. While both of these additives could be used in analytical work, their use in pilot-scale applications could be limited due to the corrosiveness and difficulty of removal after extraction (Figure 3.14).

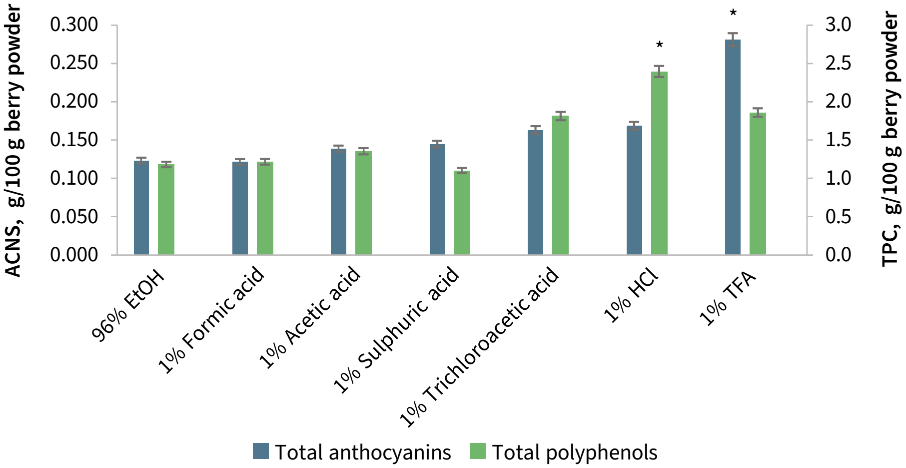


Figure 3.14. Comparison of total polyphenolic (TPC) and total anthocyanin (ACNS) extraction efficiency using various acids at the concentration of 1% with 96% ethanol (v/v). Error bars represent 95% confidence interval. Asterisk (*) represents a significant difference in the results (ANOVA, Tukey's HSD).

Selection of polyphenolic extraction method and its optimisation

Selection of an appropriate extraction method can significantly increase the amount of extracted polyphenolics and anthocyanins. To determine the method most appropriate for extraction of berry press residues and whole berries a series of extractions were done using different extraction methods, while using constant extraction solvent parameters (96% ethanol, 0.5% TFA).

The highest extraction yields were obtained using ultrasound assisted extraction, where all of the measured parameters gave the highest results. The lowest yield was achieved when using supercritical CO_2 extraction – this method has been used for extraction of polyphenolics in other studies, however, it is more appropriate for extraction of more hydrophobic substances. The effectiveness of ultrasound assisted extraction lays in the fundamental principle of this method – during the sonication process the cell walls are disrupted, thus allowing the extraction solvent to penetrate within the cell and release the cell contents into the surrounding medium. The simplicity and ease of use puts the ultrasound assisted extraction as a promising method of choice for lab-scale as well as industrial-scale applications (Table 3.12.).

Ultrasound assisted extraction provides the highest yields when extracting types of berry biomass. As one of the main parameters in ultrasound-assisted extraction the treatment duration was identified. To optimize the treatment duration a series of experiments were conducted using different concentrations of ethanol with 5% formic acid as additive. Kinetic study revealed that for both measured parameters (polyphenolics, anthocyanins) the optimal ultrasound treatment duration was 15 minutes, after which the amount of extracted polyphenolics did not increase significantly

Table 3.12. Comparison of different extraction methods. All extractions were done with 96% ethanol and 0.5% TFA, v/v. Uncertainty represents standard deviation. Different letters next to the result represent significant difference in the results ($p \leq 0.05$, Student's t-test).

Extraction method	Dry matter, g/100 g	Carbohydrates, g/100 g	Anthocyanins, g/100 g	Polyphenolics, g/100 g
Microwave	21.0 ± 0.9^{a}	8.8 ± 0.4^{a}	$0.054 \pm 0,001^{a}$	1.09 ± 0.04^{a}
Soxhlet	23.9 ± 1.8^{a}	8.3 ± 0.3^{a}	$0.065 \pm 0,002^{a}$	1.21 ± 0.05^{a}
100W ultrasound	34.1 ± 1.4^{b}	11.5 ± 0.5^{b}	$0.135 \pm 0,003^{b}$	1.59 ± 0.07^{b}
360W ultrasound	34.5 ± 1.4^{b}	12.2 ± 0.5^{b}	$0.147 \pm 0,004^{b}$	1.68 ± 0.07^{b}
Shaking (24h)	33.0 ± 1.4^{d}	11.8 ± 0.5^{d}	$0.098 \pm 0,002^{c}$	1.12 ± 0.06^{c}
Supercritical CO_2	3.41 ± 0.19^{c}	0.050 ± 0.010^{c}	ND	0.050 ± 0.010^{d}

ND – not detected or the concentration was lower than the LOD.

(Figure 3.15, 3.16). Additionally, the performed kinetics experiments demonstrated that 40% and 70% ethanol extracted more polyphenolics than 96% ethanol, indicating the optimal ethanol concentration for extraction of berry polyphenolics is in the range of 40–70%.

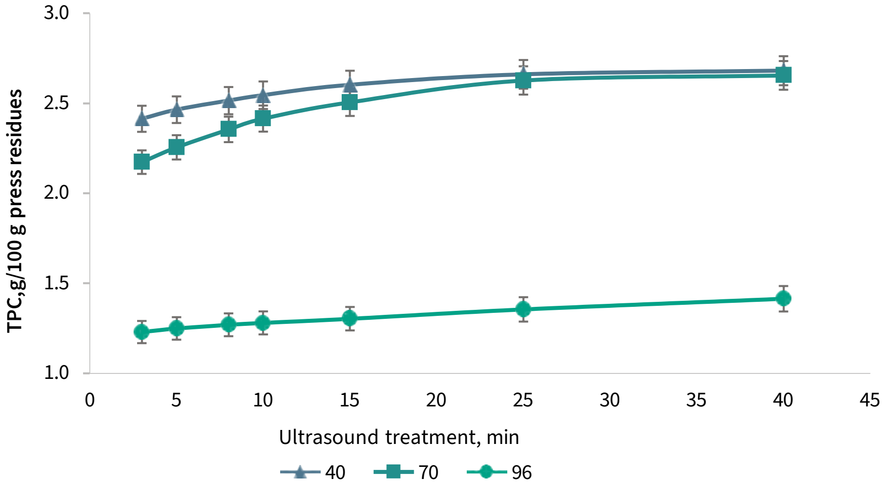


Figure 3.15. Total extracted polyphenolics from cranberry press residues depending on the duration of ultrasound treatment. Three different ethanol (%) concentrations were used with 5% formic acid (v/v). Error bars represent 95% confidence interval.

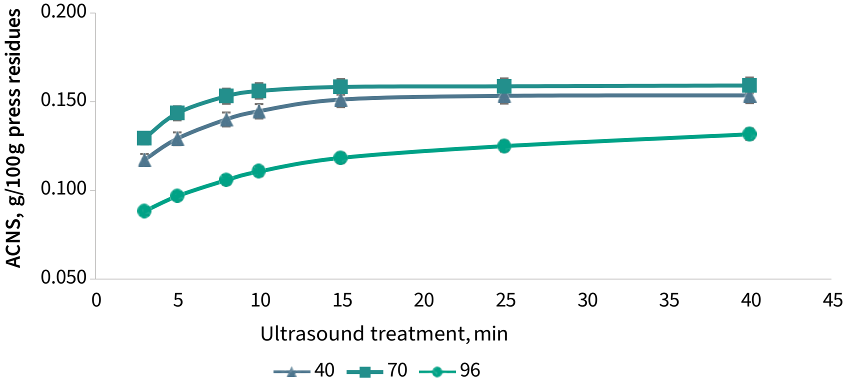


Figure 3.16. Total extracted anthocyanins from cranberry press residues depending on the duration of ultrasound treatment. Three different ethanol (%) concentrations were used with 5% formic acid (v/v). Error bars represent 95% confidence interval.

Further increasing of extraction yield can be achieved through optimisation of solvent-biomass ratio. The goal of this experiment was to decrease the amount of used solvent while reaching the maximum saturation of polyphenolics in the extract. In the experiments where ethanol was used, no significant difference could be seen between the different solid/solvent ratios. However, in the extractions where methanol was used, the optimal solid/solvent ratio was between 1:90 and 1:120, as these experiments resulted in significantly higher amounts of extracted anthocyanins (0.174 g and 0.169 g /100 g berries) and total polyphenols (2.34 g and 2.29 g /100 g berries) (Figure 3.17). Considerations must be made when using methanol over ethanol as the extraction solvent – while methanol is a more suitable solvent for use in lab-scale experiments, it should be avoided in industrial-scale applications due to its toxicity and limitations of use in the industry.

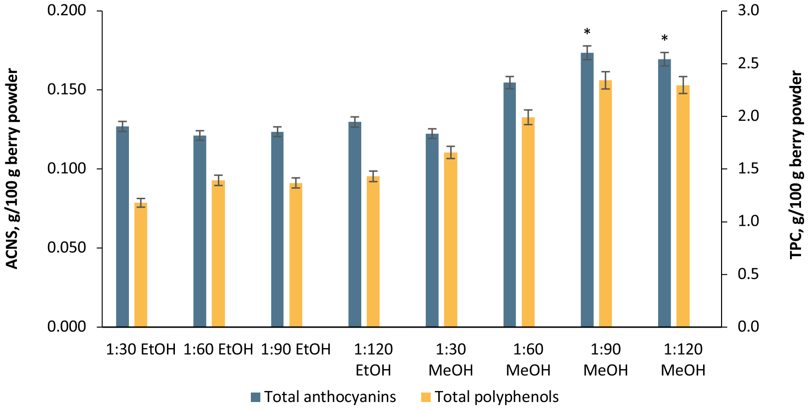


Figure 3.17. Effect of the solvent/solid ratio on the extraction of total polyphenol (TPC) and total anthocyanin (ACNS) using ethanol (EtOH) and methanol (MeOH) with 5% formic acid (v/v). Error bars represent 95% confidence interval. Asterisk (*) represents a significant difference in the results ($p \leq 0.05$, Student's t-test).

Optimisation of solvent composition for polyphenolic extraction using RSM approach

Despite the fact that the extraction of total polyphenols has been widely studied, the concentrations and compositions of extraction solvents used are still disparate. There is no consensus on the optimum extraction parameters, especially for extraction at industrial or semi industrial scales. Statistical optimisation considers the interaction between different variables and its effect on the observed response. The Response Surface Methodology (RSM) developed by Box and Wilson in 1951 uses statistical and mathematical techniques to optimise the process and identify the most significant interactions. As previously determined the main parameter in berry extraction is the extractant composition – the used solvent and the used additive (acidifying agent). Due to the diverse nature of polyphenolics, the extraction of these compounds cannot be generalised and methods of extraction must be developed for each specific group of polyphenolics found in the plant material used. The effect of ethanol or methanol together with formic acid or TFA on the extraction efficiency of total polyphenolics and anthocyanins was investigated using the RSM approach.

Ethanol and methanol both showed a similar trend when used together with TFA. The response increased with an increased concentration of TFA. The same was observed with methanol and formic acid, although they yielded the highest response when the concentration of formic acid had decreased. Aqueous ethanol (40–70%) and aqueous methanol (60–80%) are more appropriate for the extraction of total polyphenols than more concentrated solvents. The same approach was used to determine the optimal solvent mixtures for the extraction of anthocyanins. The models generated for ethanol/TFA and methanol/TFA showed that the higher the concentration of the solvent used, the more anthocyanins were extracted. The optimal concentration of ethanol for a maximum response together with formic acid was found to be 40–70%, while for methanol it was 40–60% (Table 3.13.).

The two responses examined (total anthocyanins and total polyphenols) were expected to have a strong correlation; however, the RSM approach revealed that the extraction conditions favoured by anthocyanins are not the same as those favoured by the total polyphenols. Maximisation of the extracted anthocyanins lead to a decrease of the total extracted polyphenols and vice versa. To maximise the total extracted anthocyanins and polyphenols, the extraction solvent optimal for both was chosen (Table 3.13).

Table 3.13. Solvent composition optimized using RSM approach.

Optimal conditions	Measured response	Solvent, v/v%	Acid, v/v%
	Anthocyanins	Methanol, 97.3	TFA, 0.3
		Ethanol, 40	TFA, 1
	Polyphenols	Methanol, 40	TFA, 1
		Ethanol, 90	TFA, 0.9

Optimisation of enzyme-ultrasound assisted extraction of polyphenolics using RSM approach

To facilitate the objective of bioactive compound release from berry press residues, the cell walls must rupture. Pectolytic enzymes could be used to degrade the structural polysaccharides of the berry skin cell walls, thus releasing the contents of the cells into the extraction medium. The physical characteristics of berry skins allows the use of pectolytic enzymes for the disruption of cells; however, the effects of enzymatic hydrolysis could be further improved using ultrasound. Effects of enzyme and ultrasonic treatments were evaluated and optimised using the RSM approach in order to increase the release of polyphenolic compounds (especially anthocyanins). Enzyme-ultrasound treatment was done on whole blueberries and lingonberries, the aim of these experiments was to elevate the radical scavenging activity of the prepared juice by increasing total contents of polyphenolics, anthocyanins and reduce the amount of juice production leftovers – berry press residues.

Variables to be optimised were firstly identified by performing experiments where a single factor was changed at a time. Preliminary range of extraction variables were identified – incubation temperature, length of the ultrasound treatment, amount of added ethanol before ultrasonication, pH, enzymatic incubation time. The identified extraction parameters were further optimised using RSM. In order to examine the combined effects of the previously chosen extraction variables using RSM, a central composite design of 56 runs with 4 centre points was performed randomly. The observed response variables were total polyphenolic and total anthocyanin contents (Figure 3.18).

The obtained results were used to calculate the optimal condition parameters based on the maximum extraction yields of polyphenolics and anthocyanins. Analysis on the calculated central composite design values were done to maximise the desirability of the response parameters. Similarly, to conventional solvent extraction, also enzyme assisted extraction has specific extraction condition for both of the measure variables (Table 3.14.). The minimal values obtained provided 166 mg anthocyanins/100 g, while the conditions of maximised desirability provided 1103 mg/100 g blueberry press residues. Anthocyanin yield at unoptimized conditions is only 15% of what could be obtained at the optimised conditions. The duration of ultrasound treatment after the incubation with enzymes increased the extraction yield of anthocyanins – enzyme treatment liquifies the berry skins and the cavitation caused by the ultrasound waves further breaks down the cells, thus releasing the cell contents into the extraction medium. Stability of anthocyanins depends on the pH of the medium, therefore the lower the pH used for the extraction, the higher the anthocyanin yield. While anthocyanins provide colour to the juice, which is an important factor for consumer attraction, they also have antiradical activity. Other groups of polyphenolics have even greater ability of free-radical scavenging, therefore optimisation of blueberry press residue extraction using enzyme and ultrasound treatment could provide products that have not only better visual appearance, but also increased health benefits. Compared to the optimal conditions of anthocyanin extraction, polyphenolic extraction requires longer ultrasound treatment and incubation time – this indicates that the polyphenolics are bound and incorporated into the cell membranes, as more cell degradation is necessary. The frequency of ultrasound used can also have significant influence on the enzyme activity – low sonication frequency can possibly increase activity of pectinase and other enzymes

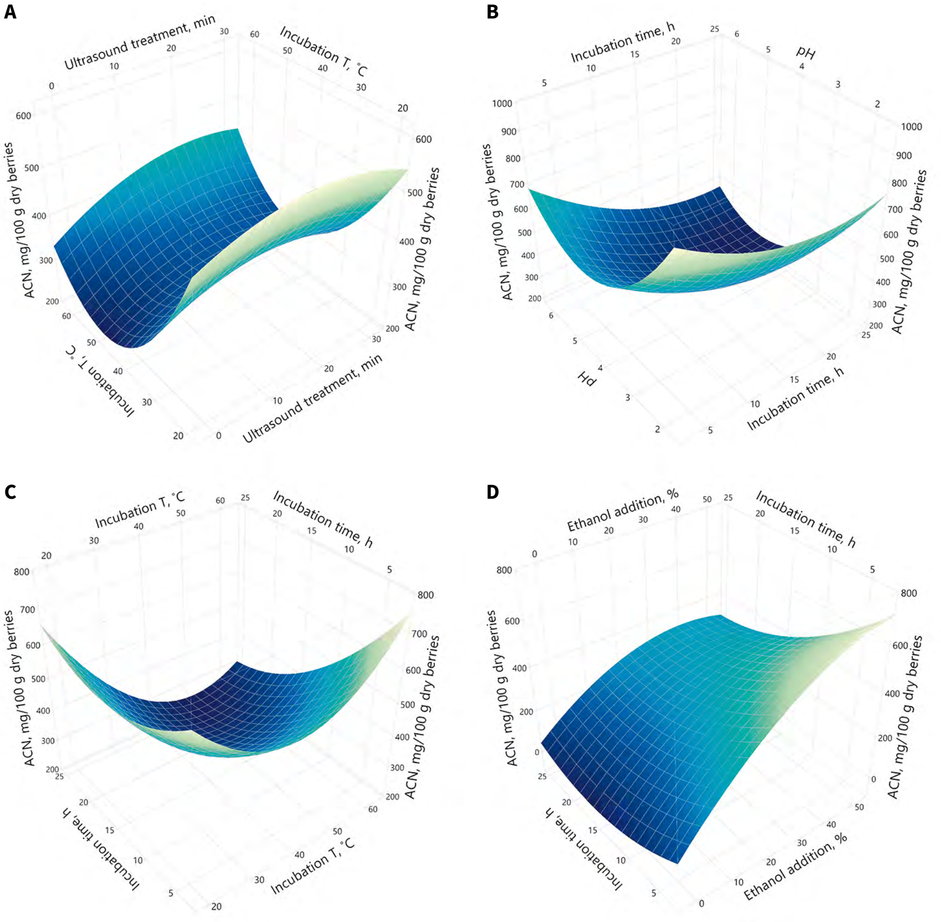


Figure 3.18. Three-dimensional response surface graphs showing the effects of the duration of ultrasound treatment (X_1), ethanol addition (X_2), incubation temperature (X_3), pH (X_4) and incubation time (X_5) on the total anthocyanin extraction yield from blueberry press residues. (A) effects of X_1X_3, (B) X_4X_5, (C) X_3X_5 and (D) X_2X_5.

that can disintegrate cell membranes thus releasing polyphenolic compounds (Bhat et al. 2011; Nguyen et al. 2013). Low frequencies of ultrasound (20–40 kHz) generate large cavitation bubbles which ensure more violent cellular degradation, thus increasing solvent penetration and extraction rate (Dzah et al. 2020). The optimal extraction duration was identified to be at the maximum (30 min) of prepared model, indicating that the true optimum is outside of the optimisation range – to decrease the extraction time and increase extraction yield, the use of ultrasound at different frequencies should be tested. Polyphenolic yield at minimal conditions is 19% of what could be obtained at the optimised conditions. Similarly, to anthocyanin extraction, also polyphenolics favour lower, more acidic extraction medium.

Table 3.14. Optimised extraction conditions of polyphenolics and anthocyanins from blueberry press residue using enzyme and ultrasound treatment.

Variable	Optimal value, polyphenolics	Optimal value, anthocyanins
Ultrasound treatment, min	30	22
Ethanol addition, %	50	50
Incubation T, °C	21	21
pH	2	2
Incubation time, h	13.3	4
Desirability	0.992	0.989

3.4.2. Qualitative and quantitative analysis of obtained polyphenolic extracts

Vaccinium berry press residue extract composition

Polyphenolic extraction yields from the investigated berries ranged from 2.44 g/100 g press residues up to 3.33 g/100 g from American cranberries and bilberries, respectively. American cranberries are large berries compared to other investigated *Vaccinium* berries, they have bright red skin and white, porous pulp, bilberries on the other hand are dark blue, purple and have highly pigmented skin and pulp. Coincidentally, the total polyphenol yields from press residues, as well as the anthocyanin contents in the extracts are higher in the dark coloured berries, blueberries, bilberries. The results presented in Table 3.15. show that each of the berries could be used to obtain specific groups of polyphenolics, for example, total procyanidins (bog cranberry) or anthocyanins (blueberries, bilberries) and possibly other groups of polyphenolics, as indicated by the total polyphenol contents in the prepared press residue extracts.

Table 3.15. Composition of *Vaccinium* berry press residue extracts.

	Polyphenol yield*	Total polyphenols**	Total procyanidins**	Anthocyanins**
American cranberry	2.44 ± 0.14	47.9 ± 2.2	0.43 ± 0.02	3.13 ± 0.14
Bilberry	3.33 ± 0.18	36. 5 ± 1.6	4.62 ± 0.21	13.3 ± 0.6
Bog cranberry	3.05 ± 0.17	48.54 ± 2.18	8.61 ± 0.39	5.95 ± 0.27
Blueberry	3.21 ± 0.18	56.0 ± 2.5	1.91 ± 0.09	34.8 ± 1. 6
Lingonberry	2.63 ± 0.15	52.5 ± 2.4	2.04 ± 0.09	1.59 ± 0.07

* – g/100 g press residues; ** – g/100 g press residue extract. Data represents means ± SD, *n*=3.

Anthocyanin composition of Vaccinium berry press residue extracts

Individual anthocyanin contents in the investigated berry press residues have been determined. In total 15 different anthocyanins have been found, 6 of which are typical for the red coloured berries (lingonberries, and both species of cranberries). The anthocyanin contents, as well as polyphenolic contents generally, depend on the quality of the berry press residues. Press residues must be kept frozen in airtight containers or bags to avoid oxidation of these substances. After a while the degradation of anthocyanins becomes apparent – the press residues start browning, which indicates the breakdown of anthocyanins. Some of the anthocyanins are more stable than others, for example, anthocyanins found in red coloured berries, are more prone to degradation. Anthocyanin profiles of each of the berries can also be used as a chemometric tool for authenticity testing, since the anthocyanin profiles of each berry are characteristic (Table 3.16.; **Article 9**).

Table 3.16. Summarized UPLC and LC-TOF qualitative and quantitative data of found anthocyanins in the studied *Vaccinium* berries.

Anthocyanins	**Amount (mg/g press residues)[a]**				
	Bilberry	**Blueberries**	**Bog cranberries**	**American cranberries**	**Lingonberry**
Delphinidin-3-*O*-galactoside	31.4 ± 3.1	7.6 ± 0.6	ND	ND	ND
Delphinidin-3-*O*-glucoside	39.7 ± 3.2	1.83 ± 0.17	ND	ND	ND
Cyanidin-3-*O*-galactoside	26.5 ± 2.3	1.56 ± 0.13	9.8 ± 1.0	1.73 ± 0.17	19.3 ± 1.9
Delphinidin-3-*O*-arabinoside	26.3 ± 2.6	6.5 ± 0.5	ND	ND	ND
Cyanidin-3-*O*-glucoside	49.2 ± 4.2	0.86 ± 0.81	0.71 ± 0.06	0.060 ± 0.010	1.65 ± 0.15
Petunidin-3-*O*-galactoside			ND	ND	ND
Cyanidin-3-*O*-arabinoside	28.7 ± 2.3	1.15 ± 0.09	9.1 ± 0.9	3.07 ± 0.31	5.44 ± 0.45
Petunidin-3-*O*-glucoside	9.0 ± 0.7	6.5 ± 0.6	ND	ND	ND
Peonidin-3-*O*-galactoside	2.73 ± 0.28	0.24 ± 0.02	12.4 ± 1.2	3.04 ± 0.21	0.33 ± 0.04
Petunidin-3-*O*-arabinoside	8.55 ± 0.76	5.5 ± 0.4	ND	ND	ND
Peonidin-3-*O*-glucoside	20.4 ± 1.9	1.58 ± 0.29	3.28 ± 0.30	0.36 ± 0.04	0.86 ± 0.07
Malvidin-3-*O*-galactoside			ND	ND	ND
Peonidin-3-*O*-arabinoside	9.9 ± 0.9	25.8 ± 2.5	8.18 ± 0.70	2.31 ± 0.23	0.31 ± 0.03
Malvinidin-3-*O*-glucoside	26.2 ± 2.4	5.3 ± 0.5	ND	ND	ND
Malvidin-3-*O*-arabinoside	6.4 ± 0.6	19.6 ± 1.1	ND	ND	ND

[a] Data are expressed as mean values ± standard deviation (n=3), ND – not detected.

Polyphenolic composition of berry press residue extracts

Individual polyphenols belonging to a variety of polyphenolic groups were determined using ORBITRAP- HRMS. Substances found were identified by comparing the found m/z to the calculated exact mass of potential candidates. Certain substances were confirmed using authentic standards, when available (Table 3.17.; **Article 10**). In total 216 signals were identified as belonging to polyphenolic substances in addition to more than 850 unidentified signals detected in the press residue extracts. The high number, diversity and lack of authentic standards limit the identification of polyphenolic compounds in such complex extracts, despite the opportunities, precision and resolution of the used high resolution mass spectrometry methods.

Table 3.17. Substances identified in the *Vaccinium* berry pomace extracts (μg/g of polyphenolic extract). Standard error of quantification data ≤5%. Values represent the means ($n = 3$). ND – not detected, concentration was lower than limit of quantification (5 μg/g).

Compound	Lingonberry	Bog cranberry	American cranberry	Bilberry	Blueberry
4-hydroxybenzoic acid	556	116	95	80	76
Protocatechuic acid	2287	1151	374	3376	2967
p-Coumaric acid	1374	313	272	182	26
Gallic acid	23	33	105	585	770
Caffeic acid	747	352	103	293	240
Ferulic acid	2352	303	33	105	454
Syringic acid	21	30	23	193	1534
Resveratrol	ND	31	73	38	50
Naringenin	73	27	14	10	16
Kaempferol	90	76	34	11	66
Cyanidin	ND	94	ND	ND	13
(+)-Catechin	14038	4368	123	53	ND
(-)-Epicatechin	3980	1632	242	1092	ND
Ellagic acid	884	345	235	207	169
Quercetin	559	893	1208	184	977
Delphinidin	1382	3408	170	1079	574
Taxifolin	1758	2285	5608	6184	1500
Myricetin	2448	2641	815	3889	3388
Malvidin	79	124	323	229	10186
Chlorogenic acid	902	10833	166	2269	145
Quercetin-3-glycoside	1020	4382	216	913	528
Procyanidin A2	22414	1893	5474	301	101
Procyanidin B2	5638	2898	48	3163	ND

Rutin	451	368	ND	ND	286
Caffeic acid hexoside	45	51	32	45	55
Coumaric acid hexoside	130	129	89	119	144
Kaempferol-3-O-ethyl-glucoside	61	73	45	69	78
Quercetin-malonyl- hexoside	53	63	31	56	68
Isorhamnetin acetylgalactoside	57	63	26	44	66
Scopoletin	39	31	30	39	44
Kaempferol acetylrhamnoside	79	87	55	80	92
Hosloppin	77	94	46	78	93

Enzyme treatment degrades certain anthocyanins

The structural polysaccharides that make up the plant cells can be degraded using pectolytic enzymes to aid the release of the cell contents into the surrounding medium. Pectolytic enzymes could be used to degrade the structural polysaccharides of the berry skin cell wall, thus releasing the contents of the cells (secondary metabolites – polyphenolics) into the extraction medium. The physical characteristics of berry skins allows the use of pectolytic enzymes for the disruption of cells, since the one of the main structural polysaccharides are cellulose, hemicellulose and pectin (Hilz et al. 2006). Anthocyanin stability after the enzyme/ultrasound treatment was evaluated using UPLC-PDA analysis (**Article 12**). In total 19 and 8 individual anthocyanins were identified in the juice samples in blueberry and lingonberry, respectively. Analysis of individual anthocyanins revealed that some of the present anthocyanins degrade due to certain enzymes (cellulases, pectinases and mixtures of the two with certain side-activities, **Article 12**). Cyanidin galactoside in lingonberry was found in both control samples, ultrasound treatment did not influence the anthocyanin contents, however, treatment with Enzyme 1, 2 and 5 significantly reduced the concentration of this specific anthocyanin. Enzyme 3 and 4 showed similar anthocyanin profiles as the untreated and ultrasound treated control samples, which indicates that these enzymes do not degrade anthocyanins, also the total anthocyanin concentrations are comparable to the control samples (Figure 3.19A). Blueberry anthocyanins, depending on the used enzyme, showed similar pattern of anthocyanin degradation – Enzymes 3 and 4 showed comparable profiles as those of control samples, while Enzymes 1,2 and 5 showed degradation of certain anthocyanins. Significant decrease of malvidin galactoside was observed after the enzyme treatment (Figure 3.19B). In lingonberries, as one of the main anthocyanins petundin-3-O-glucoside was identified. Relative amounts of certain anthocyanins significantly decreased (75% to 8% in lingonberry) as well as total amount of anthocyanins (non-treated juice 1353 mg/L; treated juice 278 mg/L) (**Article 12**).

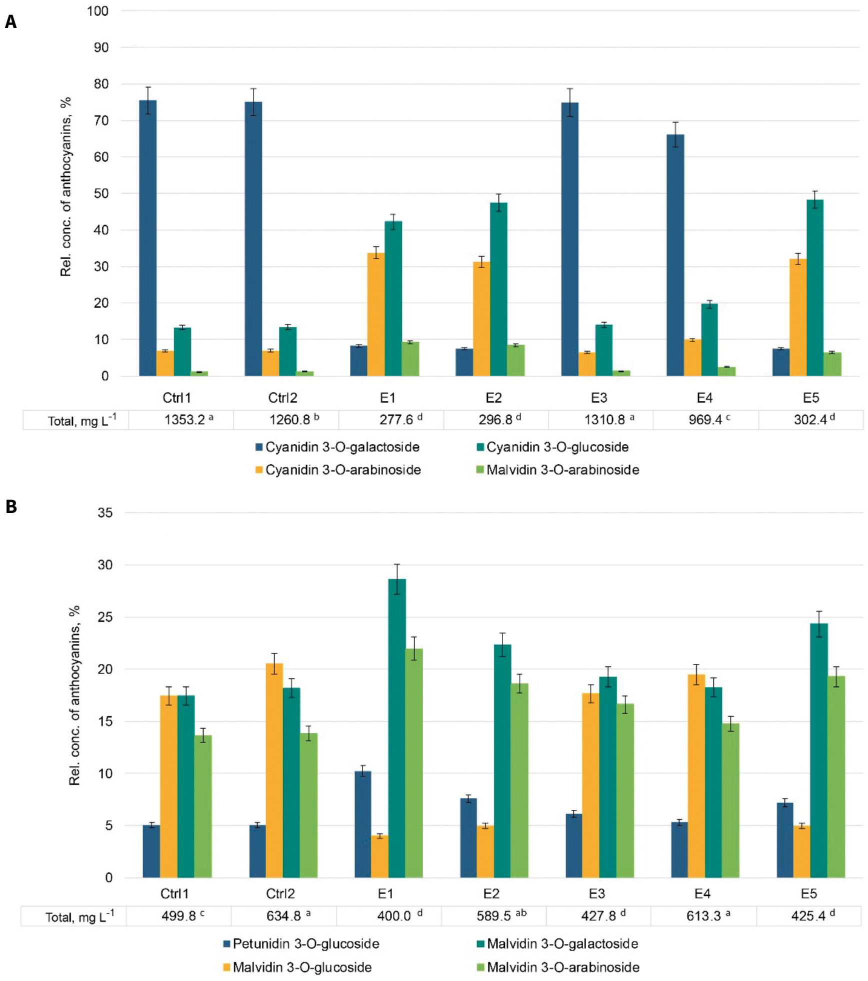


Figure 3.19. Differences in the relative concentration of main anthocyanins in (A) lingonberry and (B) blueberry juice after the enzyme and ultrasound treatment using the optimised juice extraction parameters. Error bars represent standard deviation. Connecting letters next to the Total amount of anthocyanins (mg/L) represent significant differences between the sample measurements (ANOVA, Tukeys HSD test, n=3, α=0.05).

3.4.3. Identification of polyphenolic extract applications

Optimised extraction of whole berries and berry press residues

The quantities and compositions of the extraction solvents to obtain polyphenolics still vary, despite the fact that the extraction of total polyphenols has been extensively investigated. The best extraction parameters are not universally agreed upon, particularly for extraction at industrial or semi-industrial scale. Statistical optimization takes into account how many factors interact and how that impacts the observed result.

The RSM optimizes the process and identifies the most important interactions using statistical and mathematical methods. The objective of this set of experiments was to identify the solvent compositions utilized to extract polyphenols from American cranberry press residues using RSM and to evaluate the preferred polyphenol/anthocyanin extraction conditions also for other *Vaccinium* berries and their press residues. To test the robustness of the optimized extraction conditions *Vaccinium* berries common in Northern Europe as part of the verification experiments were used. In the trials dried berry press residues were used, and entire, dried berries that had not been processed to increase the scope of the verification of the selected optimal extraction parameters. This method provided information on the polyphenolic content of both types of samples, allowing to determine whether whole berries or berry press residues have a higher potential for usage as functional ingredients and the creation of polyphenolic concentrates. Blueberry, American cranberry, and bog cranberry press residues all had a significantly more total polyphenolics than unprocessed, whole berries (**Article 9**).

The performed validation experiments showed that the optimal conditions for anthocyanin extraction gave significantly higher extraction yields compared to the control conditions (least favourable solvent composition, as determined by RSM). Press residues of several berries, like blueberries, bog cranberries, American cranberries, and lingonberries (Figure 3.20), were shown to possess more anthocyanins (w/w%) than

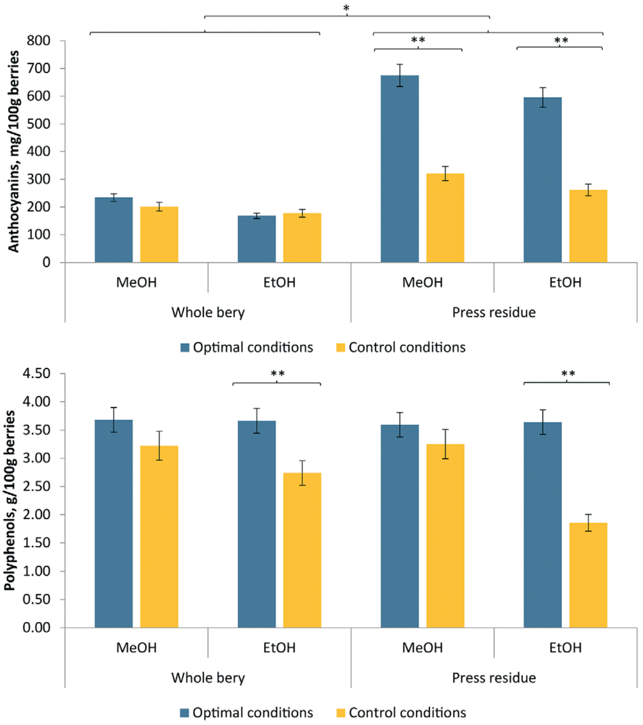


Figure 3.20. Yields of polyphenols and anthocyanins under optimal and control extraction conditions from lingonberries and their press residues using different solvents, EtOH (ethanol) and MeOH (methanol). * represents significant difference between the whole berries and press residues, ** represents significant difference between control and optimal conditions, all measurements were done in triplicate ($\alpha=0.05$, $p < 0.05$).

the whole berries do. This discrepancy can be attributed to the anthocyanins' particular distribution throughout the fruit. For instance, the dark, blue-skinned blueberry has a white or greenish pulp and almost little anthocyanins. In contrast, bilberries have anthocyanins throughout the entire berry (skin and flesh).

The aforementioned findings put the selected extraction parameters into context for use with diverse berries and sample types, both for preparative extraction of berry polyphenols and analytical work. Berry press residues were found to have higher levels of anthocyanins and total polyphenols (w/w%) than whole berries. Blueberry and American cranberry press residues, which are a waste product of juice processing, were shown to contain more polyphenols (particularly anthocyanins) per mass unit than whole berries. This establishes the press residues as a raw material for the extraction of beneficial antioxidants (**Article 9**).

Hypoglycaemic and hepatoprotective properties of berry press residue extracts

Investigated berry (bilberries, blueberries, bog and American cranberries, lingonberries) press residue extracts were tested to compare their antioxidant, hypoglycaemic and hepatoprotective properties. According to the results, bog cranberry extracts had the highest total antioxidant capacity (TAC), followed by extracts from bilberries, lingonberries, American cranberries, and blueberries. Cranberry and American cranberry extracts both have the same DPPH-scavenging abilities as ascorbic acid (control). Cranberry extracts outperformed other extracts in terms of their ability to scavenge free radicals. The bilberry pomace extract was shown to have the highest superoxide dismutase activity (SOD), but the cranberry extract had two times lower activity.

By boosting cell viability, all extracts demonstrated considerable concentration-dependent protective efficacy against tert-butyl hydroperoxide (tBH) induced cytotoxicity. Up to 0.25 mg of cranberry extract/mL exhibited improved cell viability and 100% protection. Additionally, lingonberry extract shown notable defence. All five extracts demonstrated notable HepG2 cell survival rates at 0.25 mg/mL. The best performance came from cranberry extract. Although each extract's antioxidant impact varied slightly, all five berry pomace extracts had hepatoprotective qualities.

All five berry pomace extracts had an inhibitory effect on the activities of amylase and glucosidase, with glucosidase activity being more effectively inhibited. Bilberry, bog cranberry, American cranberry, and lingonberry all had similar IC50 inhibitory values for alpha-glucosidase activity, with values ranging from 7.0 μg/mL to 16 μg/mL. Alpha-glucosidase activity was less strongly influenced by blueberry extract (IC50: 35 μg/mL). The extract potencies required to block alpha-glucosidase activity were substantially lower than those needed to block -amylase activity. The IC50 values varied between 340 and 550 μg/mL. Alpha-glucosidase activity was more efficiently suppressed by the positive reference drug acarbose in our studies than alpha-amylase. It should be emphasized that compared to acarbose (control), all berry pomace extracts demonstrated substantially higher suppression of both enzyme activity.

Overall, the findings of this study show that *Vaccinium* berry pomace extracts are effective at inhibiting the actions of enzymes involved in carbohydrate digestion. They also highlight the extracts' strong antioxidant capacity in preventing tBH-induced oxidative damage to hepatic cells at non-toxic concentrations. The presence of active polyphenols and their synergistic effects may be responsible for the extracts' hepatoprotective and hypoglycemic characteristics. Our findings promote additional research into the development of standardized formulations for the use in the prevention of chronic conditions linked to oxidative stress and the usage of berry pomace extracts (**Article 10**).

Inhibition of NF-κB pathway and COX-2 activity of berry press residue extracts

Anti-inflammatory properties of berry press residue extracts (bilberries, blueberries, bog and American cranberries, lingonberries) were tested using bacterial lipopolysaccharide (LPS)-stimulated monocytic THP-1 cells in combination with cyclooxygenase-2 (COX-2) inhibition assay.

A fluorometric enzyme inhibition experiments were used to determine the inhibitory impact of bilberry, blueberry, American cranberry, bog cranberry, and lingonberry pomace extracts on COX-2. On COX-2, all extracts had concentration-dependent inhibitory effects. More than 50% inhibition was present at 1 mg/mL, which was higher or equivalent to the control. At 0.2 mg/mL, only blueberry extract showed 50% COX-2 inhibition. At all examined concentrations examined, the bilberry extract's inhibitory impact was less than 30%.

When the cytotoxicity of berry press residue extracts was investigated, it was discovered that at concentrations of 1 mg/mL and below, the extracts did not significantly affect the viability of THP-1 cells. NF-B nuclear translocation caused by LPS was reduced by bilberry, blueberry, American cranberry, and bog cranberry extracts to the same extent as basal NF-kB nuclear translocation in unstimulated THP-1 cells. Unexpectedly, even at the lowest concentration (0.04 mg/mL), blueberry pomace extract exhibited inhibitory effects. The findings demonstrated that THP-1 cells activated by LPS released pro-inflammatory cytokines such as MMP-9, TNF-alpha, IL-beta, IL-8, and IL-23. A high enough quantity of cytokine release was produced after 1 hour of LPS treatment for conventional ELISA detection. This method can be used to show that *Vaccinium* spp. berry pomace extracts have an anti-inflammatory activity. An LPS-stimulated THP-1 cell inflammation model revealed that bilberry, highbush blueberry, American cranberry, bog cranberry, and lingonberry berry press residue extracts had anti-inflammatory effects as evidenced by the inhibition of NF-B nuclear translocation and the reduced expression of pro-inflammatory cytokines. Pomace extracts from *Vaccinium* spp. also reduced the enzyme activity of COX-2. In order to prevent non-communicable diseases brought on by inflammation, *Vaccinium* spp. berry pomace extracts may be a helpful source of bioactive components for healthy nutrition (**Article 11**).

Enzyme-ultrasound treatment produces blueberry and lingonberry juice with better properties

The optimised method was further tested to evaluate the application potential of enzyme and ultrasound treatment to prepare blueberry or lingonberry juice with increased antioxidant activity and more vibrant colour. The obtained results provide an optional method of berry press residue valorisation to produce higher quality juice or extract bioactive compounds from berry biomass. Control sample 1 was a non-treated sample, while control sample 2 was a sample treated with ultrasound. Obtained results show that the juice yield of both berries increased when ultrasound treatment was used, however, it was not significant. When an enzyme was used in combination with the ultrasound treatment, significantly higher juice yields were obtained (Table 3.18.).

Also, the dry matter of the juice increased when a combination of treatments was used, indicating release of soluble compounds into the juice. Total polyphenolics and antiradical activity increased based on the used enzyme (Table 3.18.). Larger increase, compared to control, was observed in lingonberry juice (up to 22%). This increase could be attributed to the lower pH of the juice for lingonberry (pH 3.03) than for the blueberry (pH 4.12), as it was previously concluded, that lower pH increases the activity of enzyme used as well as the stability of polyphenolic compounds. In a study where the activity of different enzymes was evaluated, it was concluded that lower pH (2–3) and lower incubation temperature significantly increases enzyme activity (Reynolds et al. 2018). Enzyme 1 showed the best results for juice production from blueberries, while enzyme 5 was more effective for processing of lingonberries (Table 3.18.). Another aspect of juice production is the visual appearance of the juice – by using the combination of the two treatments it was possible to obtain clarified, vibrant, aromatic juice. Non-treated lingonberry juice was a viscous slurry, which is difficult to press and filter, however, enzyme treatment solves these issues. To possibly increase the juice and TPC yields from blueberries, more acidic juice (for example lingonberry) could be added to the enzyme-treated biomass, in order to lower the pH. The high concentration of benzoic and other organic acids (Visti et al. 2003) in lingonberry juice lowers the pH which in turn increasing the enzyme activity, simultaneously working as natural preservatives of the juice (**Article 12**).

Table 3.18. Application of combined enzyme and ultrasound treatment for juice production from lingonberries and blueberries to obtain total polyphenolics (TPC) with high antioxidant activity (DPPH).

	Sample	Juice, mL/ 100 g berries	Juice dry matter, g /100 mL	TPC, g/L	DPPH, g TE/ L
Blueberry	Control 1	76	12.77	0.96^{a}	0.68^{a}
	Control 2	78	13.10	1.01^{b}	0.97^{b}
	Enzyme 1	85	14.00	1.14^{c}	1.20^{c}
	Enzyme 2	88	14.03	1.06^{b}	1.09^{c}
	Enzyme 3	83	13.60	1.03^{ab}	0.95^{b}
	Enzyme 4	80	13.85	0.99^{ab}	0.90^{b}
	Enzyme 5	83	14.33	1.11^{c}	1.10^{c}
Lingonberry	Control 1	56	13.06	3.40^{a}	1.34^{a}
	Control 2	64	13.29	3.34^{b}	1.36^{a}
	Enzyme 1	86	14.11	3.79^{bc}	1.42^{b}
	Enzyme 2	82	14.01	4.09^{c}	1.39^{a}
	Enzyme 3	79	14.03	3.43^{b}	1.44^{b}
	Enzyme 4	80	14.11	4.00^{c}	1.53^{c}
	Enzyme 5	80	14.83	4.36^{d}	1.66^{c}

Connecting letters next to the TPC and DPPH values represent significant differences between the measurements (ANOVA, Tukeys HSD test, n=3, α=0.05).

3.5. Analysis of stable isotope ratios and trace elements as a tool for authenticity testing and traceability

The consumption of berries from the *Vaccinium* spp., including cultivated highbush blueberries (*Vaccinium corymbosum* L.) and wild bilberries (*Vaccinium myrtillus* L.), has been steadily rising over time. Studies on the composition of these berries are therefore particularly important in light of their widespread usage in ethnomedicine, for the production of juice and jam, as functional foods, and in preparations of extracts with prospective use in the pharmaceutical and cosmetics sectors. With increasing consumption, issues such as adulteration can arise. As berries are being a niche product, especially in the case of wild, Northern berries, product authenticity becomes an issue of concern. The purpose of this study was to characterize the elemental and isotopic composition as well as variation in the concentration of elements in bilberries collected from various locations in Northern Europe as well as in commercially available blueberry samples from around the globe (Figure 3.21.). Additionally, methods to ensure the quality and authenticity of these berries have proposed. Inductively coupled plasma with optical emission spectrometry (ICPOES) was utilized to analyse the elemental composition of berries, and isotope ratio mass spectrometry (IRMS) was employed to determine the light element isotope ratio values. The findings showed that the levels of macro- and microelements in bilberries could be used to distinguish the place of origin. The relevance of IRMS technique for authenticity testing is suggested by the large discrepancies in isotope ratios between blueberries from different origins. IRMS analysis of blueberries revealed significant differences in isotope ratios based on the place of origin, indicating the possibility to use this analytical method for authenticity testing.

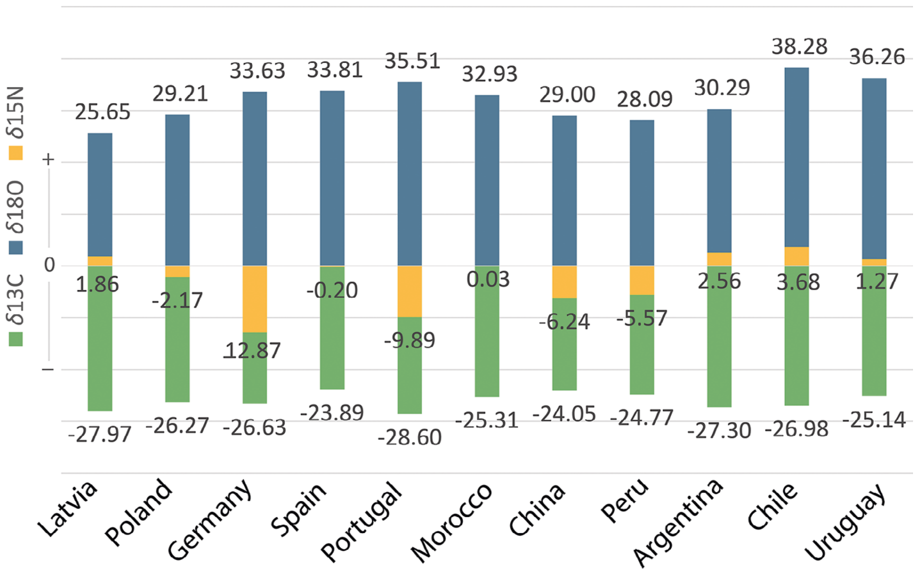


Figure 3.21. Light stable element isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) ratio values in blueberries from different countries.

The results obtained on the elemental composition of different blueberry cultivars gathered in the same field showed that the elemental composition is largely dependent on the variety of the blueberry, rather than the elemental composition of the soil. Since blueberries are grown all over the world, it was interesting to compare the elemental makeup of samples that were available for purchase. Between 2018 and 2020, in samples from nine different countries their elemental composition were examined; a total of 24 elements were analysed. The most significant quantities of K, Ca, Mg, Fe, P, S, and Mn were monitored in all examined samples. Variation among the samples was seen; for instance, the K concentration in berries from South America or North Africa was twice as high as that in samples from North Europe, where agriculture often takes place on peat soils with little mineral content. Trace element levels in commercially available cultivated berry samples were found to be low, but comparable with elemental composition reported in other studies. The larger dispersion of element concentration levels in blueberries indicates the possibility to use the elemental data for authenticity studies, however, reference values should be established first in order to tie the analysis results to certain geographical locations or geochemical conditions (**Article 13**).

Elemental composition and stable isotope ratios can be used as a tool for bilberry traceability

The bilberries were gathered across Latvia as well as in Norway, Lithuania and Finland. Bilberries in Latvia were compared to see local (within 64 000km^2) changes in elemental composition. Found elevated element concentrations indicate local or regional environmental pollution sites. For instance, elevated elemental concentrations in the Western part of Latvia indicate the presence of industrial pollution from cement production or metallurgical factories. Another major factor influencing the elemental composition of wild bilberries are the local geochemical differences of soils. The obtained results showed that the elemental variability in wild bilberries reflect a specific pattern for the territory of Latvia. Considering the geochemical conditions found in Latvia, and the general homogeneity of the soil composition, these results indicate that the elemental concentration could be used as an authenticity tool to distinguish between different regions (Table 3.19.).

Comparison of the elemental composition of bilberries sampled from the selected natural forest stands in the Baltic Sea region countries (Latvia, Lithuania and Finland) and Norway demonstrates larger variability than the nationwide sampling in Latvia (Table 3.19.). Among the studied berries from the different countries, major elements Na, Mg, P, S, B and Ba, as well as trace elements Cr and Se, were found in the highest concentrations in Norway, whereas Ni and Co were found in the lowest concentrations. Berries gathered in Finland had the lowest concentration of S, Fe, Zn and As, while the concentration of Co showed values twice as high compared to berries from other countries. Berries sampled in Lithuania had twice as high concentrations of V and Pb than in the other countries, indicating possible anthropogenic pollution near the sampling sites. The obtained results indicate geochemical specificities of element composition in berries from different regions in the Baltic Sea region and Norway. Considering the significant effect of origin on the composition of blueberries and bilberries, it is of importance to develop authentication methods for berry origins and the detection of potential adulteration.

Table 3.19. Concentrations (mg/kg) of elements in bilberry samples from Northern Europe.

Element	Latvia	Lithuania	Finland	Norway
Ca***	971±32	1020±46	1134±72	1161±56
K***	5662±66	5755±115	5083±72	5339±54
Mg***	436±34	439±43	471±14	532±28
Na***	15±4	9±3	9±3	42±2
P***	868±14	1021±12	1074±18	1234±15
S***	791±18	741±26	617±21	815±21
Al***	27.8±6.8	20.7±3,2	15.9±7.1	16.8±1.8
B***	4.5±0.4	4.8±0.7	5.2±0.5	6.3±0.3
Ba***	9.71±0.08	10.16±0.09	10.34±0.07	12.73±0.08
Cu***	4.2±0.4	5.4±0.3	3.6±0.3	3.4±0.4
Fe***	16.5±2.3	83±3	10.9±2.8	18.1±2.9
Mn***	32.7±1.1	243±12	155±21	217±18
Si***	12.7±4.7	39.6±5.9	26.7±3.2	20.9±3.9
Sr***	1.14±0.40	2.36±0.6	2.7±0.3	2.14±0.48
Zn***	6.5±0.4	7.8±0.4	5.5±0.5	7.6±0.3
As***	0.12±0.01	0.160±0.010	0.03±0.01	0.12±0.02
Cd***	0.049±0.002	0.033±0.002	0.021±0.006	0.033±0.004
Co***	0.070±0.02	0.067±0.03	0.102±0.03	0.049±0.02
Cr***	0.213±0.008	0.177±0.008	0.149±0.007	0.297±0.009
Mo***	0.073±0.002	0.091±0.002	0.281±0.002	0.096±0.002
Ni***	0.428±0.019	0.455±0.015	0.322±0.013	0.089±0.016
Pb***	0.147±0.012	0.46±0.02	0.213±0.016	0.247±0.037
Se***	0.43±0.03	0.35±0.02	0.646±0.025	0.619±0.023
V***	0.067±0.014	0.16±0.02	0.050±0.018	0.071±0.014

A nonparametric multiple test (Kruskal–Wallis) was applied with p values: ns not significant; *0.05; **0.01; ***0.001. "±" indicates SD of the measurements (*n*=9).

The use of elemental composition analyses, as well as light stable element isotope (^{13}C, ^{15}N, ^{18}O) ratio analyses, which have been previously used for the successful determination of authenticity and origin of wine and grapes, goji and other berries, could be suggested as a prospective approach for blueberries and bilberries as well. The data obtained from major and trace element and stable light isotope ratio analysis in bilberries, collected from Finland, Latvia, Lithuania and Norway, were analysed with PCA to visualise possible differences found between the regions of berry harvest (Figure 3.22.). PCA showed that each separate country produces a cluster based on the elemental and stable isotope ratio values – the neighbouring countries Latvia and Lithuania

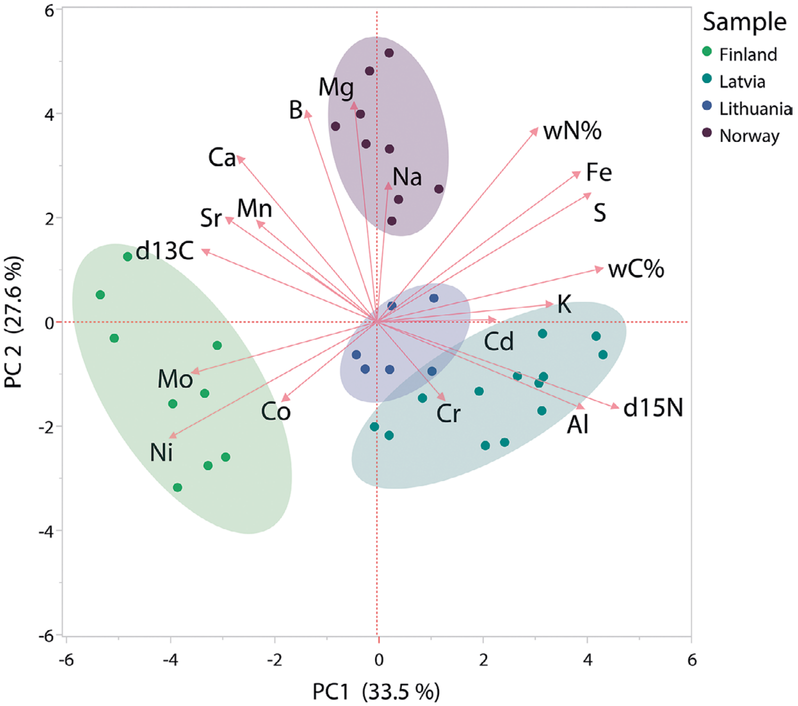


Figure 3.22. Principal components analysis of elemental and light stable isotope ratio contents in bilberries from the Baltic Sea region and Norway (n=9). Ellipses represent 95% confidence intervals.

showed slightly overlapping clusters, this is due to the similarity of soil composition. A total of 24 elements were analysed, which in combination with light stable isotope ratios allowed to differentiate between bilberries grown in countries of the Baltic Sea region and Norway. The demonstrated use of these methods could be utilised as an authenticity testing tool of berry origin (**Article 13**).

3.6. Prospects for development of food biomass processing biorefinery

This study resulted in elaboration of possible valorisation approaches for processing of *Vaccinium* berry press residues to obtain high added value products as defined by the Biomass Value Pyramid (Stegmann et al. 2020), as well as in characterization of obtained products with bioactive potential. However, obtained results can be transferred and expanded for other types of biomasses and the development can be continued to advance solutions of circular economy in the context of bioeconomy.

This study, besides the obtained results, demonstrates the relevance and potential to expand the direction presented within the PhD thesis framework beyond the discussed solutions and results. Main reasons and motivation include need for further development of biorefinery concepts of biomass processing side streams and potential to demonstrate circular economy solutions to advance bioeconomy: possibilities to use waste streams from one production as a source for other products, as well as intent to develop environmentally friendly, zero waste processing of biomass. Potential and capacity to develop further biorefinery solutions is supported also by intensive development of similar studies elsewhere, pointing at significance of continuation of efforts at development of biorefinery approaches and demonstration of their versatility in 4 outlined directions.

Direction 1. Upscaling

This study demonstrated capacity of *Vaccinium* pomace biorefinery strategy to obtain added value products. Thus, of a definite interest for many industrial applications would be upscaling of elaborated approaches to pilot and industrial scale. Upscaling of berry pomace biorefinery approaches is directly connected to Latvia's Smart Specialization Strategy which promotes bioeconomy based practices and is supported by plans to develop/increase fruit and berry cultivation, creation of new job positions in agricultural sector, orientating towards products of high added value. So far industrial scale processing of polyphenolics and lipids from berry pomaces has been elaborated to treat grape processing wastes (Farías-Campomanes et al. 2013; Cravotto et al. 2018; Gómez-Brandón et al. 2019) as well as cranberry juice production wastes (Harrison et al. 2013; Ross et al. 2017). An essential element of the upscaling phase is techno-economic analysis of production. Thus, the same principles, considering results of extraction optimisation, solvent selection and selection of other parameters could be applied also on *Vaccinium* berry press residues, supporting use of intensive and environmentally friendly extraction methods providing possibilities to obtain expected extracts with a high yield. Upscaling could be done using berry pomace processing modular approach: concentrating on one group of substances (extracts), for example, oils, polyphenolics, procyanidins or other groups of extracts. However, in this case unavoidably significant volumes of secondary wastes will be generated. This should be considered at the development of chemical processing technologies. Thus, elaborating *Vaccinium* berry pomace processing strategies, environmental considerations should be made and preference should be given to integrated processes for the comprehensive utilization of pomace by the production of multiple value-added products and evaluation of production economic feasibility at a commercial scale moving towards zero waste technologies. Feasibility of such approach has been convincingly demonstrates by Jin (2020) and Sirohi et al. (2020) on example of grape pomace processing technology. Considering results obtained within this study, elaborated approaches in these studies, there are excellent prospects to develop these methods accordingly to the needs and production capacities for *Vaccinium* berry press residue processing technologies.

Direction 2. Biorefinery

Biorefinery of biomass processing side streams is one of the hot topics of research attracting researchers from many fields, resulting in new theoretical concepts, based mostly with new applications (Stegman et al. 2020). The work presented in this PhD thesis is contributing to the development of biorefinery and proposing new solutions for *Vaccinium* berry press residue processing. At the same time there are several highly prospective directions of research illustrating prospects to develop biorefinery strategies as well as methods used, as it has been shown on other biomass examples. At first new extraction methods could be applied and known ones studied, to reach industrial application aims and major direction of efforts are oriented towards wider implementation of green processing methods. An evident actuality is orientation towards zero waste biorefining extraction approaches (Kitrytė et al. 2020), addressing use of green solvents (Dienaite et al. 2021) and expanding use of supercritical, pressurized, enzyme assisted extraction methods (Mackela et al. 2015). One of major tasks of developments of extraction is to achieve separation of different substance groups already during extraction stage (Vigano et al. 2017; Tamkute et al. 2020) also applying modelling tools (Santana et al. 2019). These few examples of ongoing research and directions with most promising potential to achieve progress, demonstrates possibilities to further advance biorefinery methods and develop new products with lower impact on the environment.

Direction 3. Fibres

By mass a significant component of *Vaccinium* berry press residues are their fibres composing 50–70 % of pomace mass (Hotchkiss et al. 2021; Jagelaviciute et al. 2022) – skins, seeds, fragments of cell structure etc. *Vaccinium* berry fibres are composed of polysaccharides and oligosaccharides of pectin, lignin, cellulose, hemicellulose, and inulin. Attached to the fibre polysaccharides valuable compounds like polyphenolics, procyanidins, proteins and small amounts of lipids are bound (Hussain et al. 2022). Depending on solubility fibres are described as soluble and insoluble. *Vaccinium* berry fibre polysaccharides are composed on xyloglucan, arabinoxylan, mannan and other units and thus do not contribute to energy production processes in human body (Hotchkiss et al. 2021). Presence of fibres in human diets is highly significant as they have major positive impacts on human health, such as reduction of risks of cardiovascular diseases (coronary heart disease, stroke, hypertension), gastrointestinal disorders, obesity and other diseases (Hussain et al. 2022). Considering this, *Vaccinium* berry pomace is highly promising product for food industries and this application possibility has a strong position accordingly to Biomass Value Pyramid concept. The potential of *Vaccinium* berry fibre has been demonstrated on several examples, using them as additives to sweet bakery products, cookies (Quiles et al. 2018), functional foods (Perez et al. 2018), extruded cereal-based food (Rohm et al. 2015) and other applications. Considering the fibre composition, as their source fibre itself after drying of pomaces and milling, as well as pomace residues after extraction of lipids and polyphenolics can be used. In the latter case insoluble fibres will dominate. The potential to use depleted fibre has been demonstrated using fibre obtained as pectin rich fraction after wine making (Feng et al. 2019) as well as enzymatically modified cranberry pomace (Jagelaviciute et al. 2022). As an important element of elaboration of research planning and development of properly functioning technology, market analysis, material flow cost accounting, carbon footprint calculation as well as life cycle cost analysis are of importance, but as demonstrate by Rohm et al. (2015), use of berry pomace fibre is highly promising, thus motivating to apply this approach also on *Vaccinium* berry press residues and residues after their extraction in the biorefinery process primary phases.

Direction 4. Zero-waste

As the final step to develop zero waste (*Vaccinium* berry press residue full utilization) biorefinery approach complete treatment of residues after biomass processing must be developed. Technically, considering costs, the simplest approach is composting of residues after extraction, as it has been demonstrated on example of composting of grape pomaces (Salgado et al. 2019). However, *Vaccinium* berry pomaces and residues after their extraction can be acidic, thus the value of obtained compost is low (Cox and Lopes 2008) and composting is a climate positive process which releases greenhouse gasses. Considering the low calorific value and limited possibilities to use berry pomace after extraction as most prospective waste-to-energy approaches can be considered.

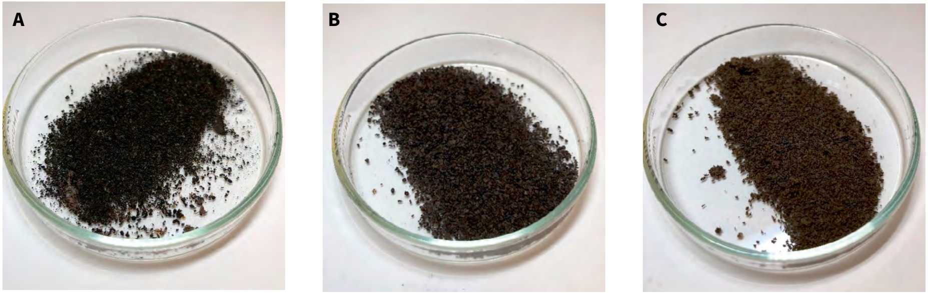


Figure 3.23. Hydrochar prepared from cranberry press residues at different pH. Acidic conditions (pH 2) (A), neutral conditions (pH 7) (B) and alkaline conditions (pH 11) (C).

Two methods could be applied, as already suggested for multiple biomass residue types (González-Vázquez et al. 2018; Duman et al. 2018; Jin et al. 2021): 1) processing of waste after ethanol extraction to obtain syngas, biogas or biohydrogen by the use of gasification or fermentation; 2) pyrolysis to obtain biochar. Both of these technologies can be applied also on *Vaccinium* berry press residues or their residues after biorefinery steps, however as criteria for method selection volumes of wastes to be processed should be considered. Gasification suggests the use of continuous process and rational use of produced biogas and thus gasification technologies are in favour for large-scale, year-round biomass waste processing. For small and medium scale complete processing of press residues and their processing residues as relevant and promising seems to be thermal treatment of unusable wastes accordingly to 2 approaches (Pala et al. 2014; Akarsu et al. 2019): 1) hydrothermal carbonization of wet biomass at temperatures 180–240 °C to obtain hydrochar and artificial humic substances (Figure 3.23.); 2) use of pyrolysis to produce the biochar at temperatures up to 800 °C. Potential of both methods for processing of biomass wastes, including cranberry pomace has been demonstrated and this direction of research deserves to be expanded (Klavins et al. 2021; Ansone-Bertina et al. 2022). Both hydrochar and biochar can find application as soil amendments and components of substrates with long life-time, thus making the waste carbonization methods as carbon neutral or even carbon negative, when all of the processing steps are implemented within the scopes of bioeconomy (Yrjälä et al. 2022). Concluding, there is a great potential for *Vaccinium* berry press residue processing as zero waste technology, returning spent biomass wastes to their cultivation areas or at least partly covering needs in energy used for their processing.

Conclusions

1. A precondition, to reach the aims of bioeconomy, is elaboration of biomass (accordingly to circular bioeconomy concept preferably using biomass waste) processing approaches (strategy), considering the composition, application possibilities of obtained substances and materials and market needs. Thus, in-depth analysis of biomass composition determines the direction of biorefinery approach development and identification of position in biorefinery value pyramid as well as support elaboration of processing methods as a key factor affecting the success of biomass biorefinery possibilities.
2. Berry lipids are from the point of application potential one of the most valuable groups of compounds supporting health promoting value of berries and can play a significant role in new, user friendly, innovative product development. Study of *Vaccinium* berries, their press residues and their berry lipid extraction possibilities reveal major dependence of extraction yields and extract composition based on the used extraction solvent, preferable use of intensive extraction methods primarily using environmentally friendly extraction solvents. The proposed extract fractionation approach demonstrates potential to obtain lipid groups with functionally applicable composition, thus widening lipid extract processing possibilities. The broad spectrum of compound groups found in the berry lipids make them attractive as antibacterial agents for various applications in food preservation, preventive aids, as part of nutraceuticals, cosmetics and could possibly reduce the use of conventional antibiotics. The sun protection factor values obtained show that the use of berry lipids have a potential application in sunscreen production, thus substituting synthetic and inorganic sunscreen constituents to natural and sustainable components.
3. Study of epicuticular and bulk waxes of studied berries and their press residues resulted in identification of major components of wax pool and supported development knowledge on wax as a significant element of plant protective mechanisms against stress. Wax composition analysis using different methods revealed epicuticular wax morphology, component variability depending on geographical and climatic factors, indicating potential plant reactions on climate change impacts. In-depth study of berry wax composition and demonstration of environmentally friendly extraction possibilities supporting application possibilities of wax derived from berry juice production waste.
4. In-depth study of *Vaccinium* berry and their press residue polyphenolics demonstrated versatility and uniqueness of their pool in comparison with other plants and significance for development of functional ingredients or products for application. Optimisation of polyphenolic group extraction compositions, application of Response Surface Methodology as well as enzymatically assisted extraction provided possibility to obtain specific polyphenolic groups with high yield using environmentally friendly extraction systems, further fractionation resulted in identification of new polyphenolics previously not described as part of *Vaccinium* berries that have high biological (pharmacological) activity as demonstrated by their radical scavenging capacity, anti-inflammatory, hepatoprotective, hypoglycemic and other activities.
5. Analysis of berry elemental composition, determination of light, stable isotope ratios, as well as analysis of berry and their press residue lipid and polyphenolic

compositions support possibilities to identify their origin – achieving authentication and traceability tasks.

6. Complete, zero-waste strategy could be elaborated by using the proposed biorefinery approaches in addition to plausible biomass conversion procedures, that have been successfully demonstrated and used for other types of high-volume food industry wastes. Specific groups of compounds could be retrieved from the food wastes and the spent biomass could further be transformed into energy or the carbon held in the specific biomass could be sequestered and used in agriculture to achieve climate neutrality or even negativity, thus elaborating circularity principles.

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Appendices – article depository

ARTICLE 1

Gas chromatography–mass spectrometry study of lipids in northern berries
L. Klavins, J. Kviesis, I. Steinberga, L. Klavina and M. Klavins
Agronomy Research, 14, 1328–1346

ARTICLE 2

Lipids of cultivated and wild *Vaccinium* spp. berries from Latvia
L. Klavins, A. Viksna, J. Kviesis, M. Klavins
Proceedings of the 13th Baltic Conference on Food Science and Technology, 198–203

DOI: 10.22616/FoodBalt.2019.019

ARTICLE 3

Composition, sun protective and antimicrobial activity of lipophilic bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.) extract fractions
L. Klavins, M. Mezulis, V. Nikolajeva, M. Klavins
LWT – Food Science and Technology, 138, 110784

https://doi.org/10.1016/j.lwt.2020.110784

ARTICLE 4

Compositional and morphological analyses of wax in northern wild berry species

P. Trivedi, K. Karppinen, L. Klavins, J. Kviesis, P. Sundqvist, N. Nguyen, E. Heinonen, M. Klavins, L. Jaakola, J. Väänänen, J. Remes, H. Häggman

Food Chemistry, 295, 441–448

https://doi.org/10.1016/j.foodchem.2019.05.134

ARTICLE 5

Cuticular wax composition of wild and cultivated northern berries

L. Klavins, M. Klavins

Foods, 9 (5), 587

https://doi.org/10.3390/foods9050587

ARTICLE 6

Analysis of composition, morphology, and biosynthesis of cuticular wax in wild type bilberry (*Vaccinium myrtillus* L.) and its glossy mutant.

P. Trivedi, N. Nguyen, L. Klavins, J. Kviesis, E. Heinonen, J. Remes, S. Jokipii-Lukkari, M. Klavins, K. Karppinen, L. Jaakola, H. Häggman

Food Chemistry, p.129517

https://doi.org/10.1016/j.foodchem.2021.129517

ARTICLE 7

Temperature has a major effect on the cuticular wax composition of bilberry (*Vaccinium myrtillus* L.) fruit

P. Trivedi, L. Klavins, A. L. Hykkerud, J. Kviesis, D. Elferts, I. Martinussen, M. Klavins, K. Karppinen, H.M. Häggman, L. Jaakola

Frontiers in Plant Science, p.3497

https://doi.org/10.3389/fpls.2022.980427

ARTICLE 8

Comparison of methods of extraction of phenolic compounds from American cranberry (*Vaccinium macrocarpon* L.) press residues

L. Klavins, J. Kviesis, M. Klavins

Agronomy Research, 15(2), 1316–1330

ARTICLE 9

Berry press residues as a valuable source of polyphenolics: Extraction optimization and analysis

L. Klavins, J. Kviesis, I. Nakurte, M. Klavins

LWT – Food Science and Technology, 93, 583–591

https://doi.org/10.1016/j.lwt.2018.04.021

ARTICLE 10

Antioxidative, hypoglycaemic and hepatoprotective properties of five *Vaccinium* spp. berry pomace extract

R. Muceniece, L. Klavins, J. Kviesis, K. Jekabsons, R. Rembergs, K. Saleniece,
Z. Dzirkale, L. Saulite, U. Riekstina, M. Klavins
Journal of Berry Research, 9(2), 267–282

10.3233/JBR-180351

ARTICLE 11

Inhibition of NF-κB pathway in LPS-stimulated THP-1 monocytes and COX-2 activity in vitro by berry pomace extracts from five *Vaccinium* species

L. Kunrade, R. Rembergs, K. Jekabsons, L. Klavins, M. Klavins,
R. Muceniece, U. Riekstina
Journal of Berry Research, 10(3), 381–396

10.3233/JBR-190485

ARTICLE 12

Optimisation of blueberry (*Vaccinium corymbosum* L.) press residue extraction using a combination of pectolytic enzyme and ultrasound treatments.

L. Klavins, E.P. Puzule, J. Kviesis, M. Klavins
Journal of Berry Research, 12(1), 41–57

10.3233/JBR-210722

ARTICLE 13

Trace Element Concentration and Stable Isotope Ratio Analysis in Blueberries and Bilberries: A Tool for Quality and Authenticity Control

L. Klavins, I. Maaga, M. Bertins, A.L. Hykkerud, K. Karppinen, Č. Bobinas, H. M. Salo, N. Nguyen, H. Salminen, K. Stankevica, M. Klavins

Foods, 10(3), 567

https://doi.org/10.3390/foods10030567

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LINARDS KĻAVIŅŠ

VACCINIUM ĢINTS OGU UN TO SPIEDPALIEKU BIORAFINĒŠANA BIOLOĢISKI AKTĪVU, FUNKCIONĀLU SASTĀVDAĻU IEGŪŠANAI

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Anotācija

Ogu sulas pārstrādes procesā veidojas būtiski atkritumu daudzumi – ogu spiedpaliekas, kas sastāv no ogu mizām un sēklām un potenciāli satur vērtīgus savienojumus, kas varētu tikt izmantoti citās nozarēs. Izmantojot biorafinēšanas principus, iespējams piešķirt pievienoto vērtību šiem atkritumproduktiem, iegūstot ekstraktus vai frakcijas ar specifisku pielietojumu. Šā promocijas darba mērķis bija izvērtēt lipīdu un polifenolu atgūšanas iespējas no ogu spiedpaliekām un veikt atgūto savienojumu izmantošanas iespēju izpēti. Ogu lipīdi, tostarp virsmas vaski, satur daudzus lipofīlus savienojumus ar dažādām funkcijām. Lipīdu ekstraktiem tika pierādīta antimikrobiālā aktivitāte un saules aizsardzības īpašības, atbalstot lipīdu ekstraktu un to frakciju pielietojumu kosmētikā. Tika optimizēta polifenolu un antociānu ekstrakcija, izmantojot atbildes virsmas metodi, kas ļauj iegūt maksimāli augstu ekstrakcijas iznākumu. Iegūtie attīrītie polifenolu ekstrakti tika analītiski raksturoti, un to izmantošana tika novērtēta, lietojot dažādus *in vitro* šūnu testus. Rezultāti par lipīdu un polifenolu sastāvu ogās un to spiedpaliekās var tikt izmantoti kā rīks, lai noteiktu ogu autentiskumu un izcelsmes vietu.

Atslēgas vārdi: *Vaccinium* ogas, lipīdi, polifenoli, pielietojums, valorizācija, biorafinēšana.

Saturs

Saīsinājumi

ACN – antocianidīni, kopējais antocianidīnu daudzums
BSTFA – N,O-bis(trimetilsilil)trifluoracetamīds
DPPH – 2,2-difenil-1-pikrilhidrazils
GC-MS – gāzes-šķidruma hromatogrāfija – masas spektrometrija
HPLC-PDA – augstas izšķirtspējas šķidrumu hromatogrāfija ar fotodiožu matricas detektoru
ICPOES – induktīvi saistītās plazmas optiskās emisijas spektrometrija
IRMS – izotopu attiecības masas spektrometrija
PCA – galveno komponentu analīze
RSM – atbildes virsmas metodoloģija
SCO_2 – oglekļa dioksīds superkritiskā stāvoklī
SPF – saules aizsardzības faktors
TE – troloks ekvivalents
TFA – trifluoretiķskābe
TPC – kopējais polifenolu daudzums
UPLC – īpaši augstas veiktspējas šķidrumu hromatogrāfija
UV/VIS – ultravioletais/redzamais starojums

Ievads

Atkritumi kā rūpnieciskās ražošanas un cilvēku patēriņa blakusprodukts ir viena no lielākajām mūsdienu problēmām. Pārtikas ražošanas zudumi un atkritumi veido apmēram 30 % no visiem atkritumiem, kas ir vērtīgu resursu zudums, vienlaikus radot arī vides problēmas (FAO 2019). Esošās pārtikas ražošanas, patēriņa un atkritumu apsaimniekošanas pieejas acīmredzami neatbilst ilgtspējīgas attīstības principiem. Pārtikas atkritumu un organisko atkritumu apsaimniekošanas problēma kļūst vēl aktuālāka, ņemot vērā mērķi atteikties no fosilajiem materiāliem un tajos balstītas ražošanas un veicināt uz bioloģiskiem resursiem balstītu ekonomiku – bioekonomiku (EK 2018), panākot klimatneitrālu un resursus taupošu attīstību (Green Deal 2019). Tomēr, lai īstenotu pāreju uz bioekonomiku, ir nepieciešams daudz vairāk zināšanu un inovāciju attiecībā uz materiālu īpašībām un ražošanu, atkritumu apsaimniekošanu un pārstrādi. Atslēgas vārdi, kas saistīti ar bioekonomikas progresu, ir biorafinēšana, valorizācija un aprites ekonomika.

Ogas, augļi un dārzeņi ir vieni no visplašāk patērētajiem pārtikas produktiem, un to pārstrāde ir saistīta ar lielu atkritumu plūsmu rašanos. Pārtikas atkritumi rodas, ražojot dažādus produktus, piemēram, sulas, sulu koncentrātus, konservētus un dehidrētus augļus un ogas, ievārījumus un citus (Campos et al. 2020). Piemēram, pasaulē gadā tiek saražoti 366 miljoni tonnu ābolu, un, tos pārstrādājot sulā, paliek 3–4,2 miljoni tonnu ābolu spiedpalieku, ko sauc arī par izspaidām (FAO 2019).

Arvien populārākas kļūst ogas, un to vidū ir arī *Vaccinium ģints* ogas (dzērvenes (*Vaccinium oxycoccos* L.), lielogu dzērvenes (*Vaccinium macrocarpon* L.), krūmmellenes (*Vaccinium corymbosum* L.), meža mellenes (*Vaccinium myrtillus* L.), brūklenes (*Vaccinium vitis-idaea* L.) un zilenes (*Vaccinium uliginosum L.),* kas ir tradicionāla uztura sastāvdaļa Latvijā, kā arī citās Ziemeļeiropas valstīs. Mūsdienās interese par *Vaccinium ģints* ogu fitoķīmisko sastāvu ir ievērojami palielinājusies, atspoguļojot sabiedrības interesi par dabisku un veselīgu pārtiku, līdz ar to pieaugusi arī interese par ogu sastāvu un labvēlīgo ietekmi, veselību ietekmējošiem faktoriem (Nile and Park 2014). Savvaļas un kultivētās *Vaccinium* ogas kļūst par plaši izplatītiem patēriņa produktiem, tāpēc to sastāva pētījumi paplašinās, lai uzlabotu esošo un attīstītu jaunu ogu, to pārstrādes produktu un ekstraktu pielietojumu. Ogu spiedpaliekas satur lielu daudzumu bioaktīvo savienojumu ar atbilstošu ķīmisko sastāvu un uzturvērtību. Ogas satur lipīdus, polifenolus, šķiedrvielas un citas vielas un var kalpot kā lielisks vērtīgu sastāvdaļu avots pārtikas rūpniecībai, veselībai, kosmētikai. No šā viedokļa ne tikai veselas ogu, bet arī to spiedpalieku pētījumi (ekstrakcija un fitoķīmiskā analīze) ir svarīgi un kļūst arvien aktuālāki, ņemot vērā dabiskas produkcijas pieprasījumu un vajadzību pēc rūpniecības izejvielām. Vienlaikus jāatzīmē, ka, neraugoties uz *Vaccinium* ģints ogu izpētes aktualitāti, izpratne par ogu, to spiedpalieku sastāvu un to izmantošanas potenciālu ir nepietiekama un nenodrošina biorafinēšanas metožu izstrādi un praktisku pielietojumu, lai sekmētu aprites bioekonomikas attīstību.

Promocijas darba mērķis

Promocijas darba mērķis ir pētīt Ziemeļeiropā sastopamo *Vaccinium* ģints ogu sastāvu, to pārstrādes atkritumproduktu (ogu spiedpalieku) biorafinēšanas risinājumus, lai sekmētu ogu sastāvā esošo savienojumu izmantošanas iespējas bioekonomikā.

Hipotēze

Pārtikas ražošanas atkritumprodukti (ogu spiedpaliekas) satur dažādas bioloģiski aktīvu vielu grupas, kuras var izdalīt, izmantojot videi draudzīgas pārstrādes (biorafinēšanas) metodes, lai iegūtu to funkcionālos komponentus ar izmantošanas potenciālu pārtikas ražošanā, kosmētikā, vienlaikus nodrošinot ogu izcelsmes autentiskuma pierādīšanas iespējas.

Promocijas darba uzdevumi

1. Ekstrakcijas, frakcionēšanas, attīrīšanas un paraugu žāvēšanas metožu izpēte, kas izmantojamas veselu ogu un ogu spiedpalieku polifenolu un lipīdu ekstraktu iegūšanai.
2. Videi draudzīgu bioloģiski aktīvo vielu ekstrakcijas metožu optimizācija no *Vaccinium* ģints ogām un to spiedpaliekām saskaņā ar biorafinēšanas principiem.
3. Polifenolu ekstrakcijas metožu izstrāde no ogu spiedpaliekām, izmantojot intensīvas ekstrakcijas metodes.
4. Pētīto ogu polifenolu un lipīdu ekstraktu sastāva izpēte.
5. *Vaccinium* ģints ogu un to spiedpalieku bioloģiskās, farmakoloģiskās un citu aktivitāšu izpēte, lai identificētu potenciālās pielietošanas jomas.

Promocijas darba zinātniskā novitāte

1. Ekstrakcijas metožu izstrāde un optimizācija polifenolu ekstrakcijai no *Vaccinium* ģints ogām un to spiedpaliekām.
2. Lipīdu ekstrakcijas metožu izstrāde no *Vaccinium* ģints ogām un to spiedpaliekām.
3. *Vaccinium* ģints ogu un to spiedpalieku polifenolu un lipīdu sastāva izpēte: jaunu to sastāvā ietilpstošu savienojumu identifikācija un kvantifikācija.
4. Ogu un to spiedpalieku ekstraktvielu frakcionēšanas metožu izstrāde, vadoties pēc to sastāvā esošo savienojumu bioloģiskās aktivitātes.
5. *Vaccinium* ģints ogu un to ekstraktu autentiskuma noteikšanas metožu izstrāde.

Pētījuma lietišķā nozīme

1. Pārtikas ražošanas atkritumu veida (ogu spiedpaliekas pēc sulas ieguves) biorafinēšanas pieejas izstrāde: videi draudzīgu ekstrakcijas metožu izstrāde.
2. *Vaccinium* ģints ogu ekstraktvielu izmantošanas potenciāla pierādīšana.
3. Ogu ekstraktvielu pielietojuma prototipu izveide.

Rezultātu aprobācija

Promocijas darba rezultāti publicēti 13 zinātniskajos rakstos ($h = 8$, 189 citāti; 01.02.2023. *Scopus*), kopumā promocijas darba autoram ir 25 zinātniskās publikācijas. Darba rezultāti ir prezentēti 18 starptautiska un vietēja mēroga konferencēs.

Ar promocijas darbu saistītās zinātniskās publikācijas

Šajā sarakstā iekļautie raksti ir izmantoti promocijas darba sagatavošanā. Raksti ir numurēti atbilstoši to tēmai (1. attēls) un pēc parādīšanās rezultātu sadaļā, turpmāk tekstā uz tiem atsaucas kā uz **1.**, **2.**, **3.** utt. **publikāciju**. Šajā darbā iekļautie raksti ir indeksēti SCOPUS un Web of Science datubāzēs un pieder pie attiecīgo izdevumu Q1 un Q2 kvartilēm. Pilnus rakstus var atrast, skenējot QR kodus disertācijas beigu sadaļā

“Pielikumi – rakstu krātuve” vai pēc to attiecīgajiem DOI, kas atrodami tajā pašā sadaļā, kā arī iepriekš minētajās zinātniskajās datubāzēs.

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Intelektuālā īpašuma aizsardzība

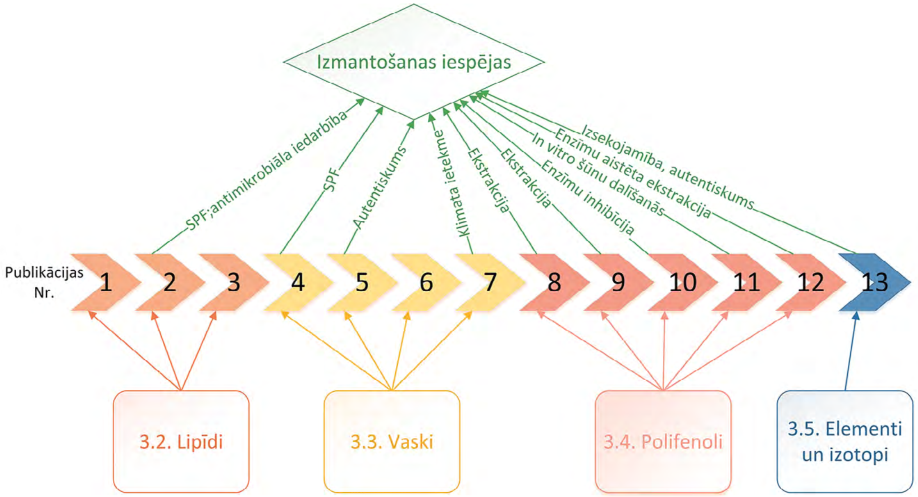
14.01.2020. piešķirts patents (Nr. 15504) izgudrojumam *“Paņēmiens polifenolu iegūšanai un attīrīšanai”.*

Autora ieguldījums disertācijas izstrādē

Linards Kļaviņš ir veicis dažādu šajā darbā pētāmo ogu paraugu ievākšanas plānu sagatavošanu, paraugu vākšanu no paraugu ņemšanas vietām visā Latvijā, kā arī izveidojis paraugu vākšanas tīklu citās valstīs (Norvēģijā, Zviedrijā, Somijā, Lietuvā), paraugu sagatavošanu ekstrakcijai un analīzei, kā arī pats veicis dažādas analīzes. Autors ir izstrādājis pētījumu plānus, ieskaitot veikto eksperimentu eksperimentālo plānojumu, paraugu analītisko raksturošanu, iegūto datu statistisko analīzi, datu vizualizācijas un aprakstu.

Darba struktūra

Promocijas darbs ir sadalīts 5 apakštēmās, kuras ir atspoguļotas vairākos publicētajos zinātniskajos rakstos. 1.–3. publikācijā ir aplūkoti ogu lipīdi, 4.–7. publikācijā – ogu vaski, 8.–12. publikācijā – ogu polifenoli, 13. publikācijā – mikroelementi un stabilo izotopu saturs (1. attēls).



1. attēls. Disertācijā publicēto zinātnisko rakstu galvenās tēmas. Publicēto zinātnisko rakstu tēmu par ogu lipīdiem (oranžā krāsā), ogu vasku (dzeltenā krāsā), polifenoliem (sarkanā krāsā), metāliem un stabiliem izotopiem (zilā krāsā) un to potenciālajām pielietošanas jomām (zaļā krāsā) saistība.

1. LITERATŪRAS APSKATS

1.1. Pārtikas atkritumu problēma un biorafinēšana kā rīks tās risināšanai

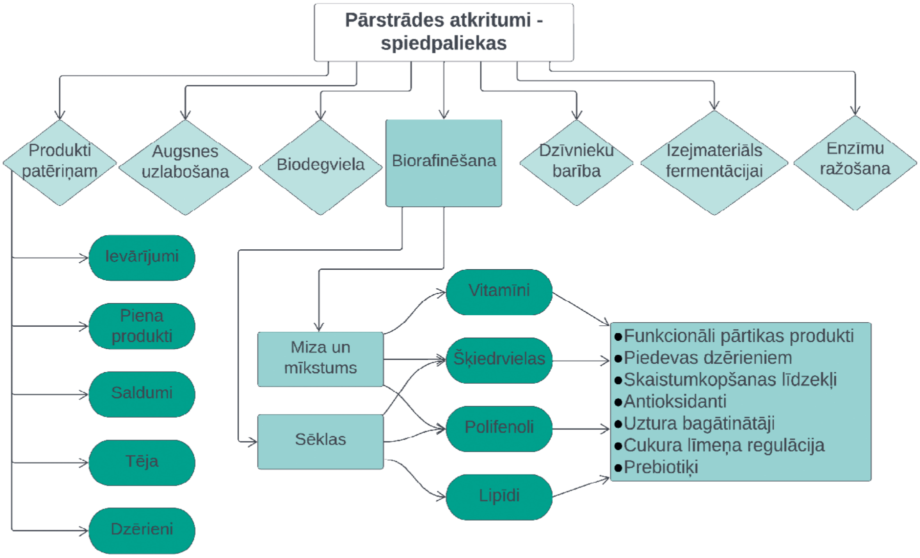
Lai sasniegtu ilgtspējīgas attīstības mērķus, viens no priekšnoteikumiem ir racionāla resursu izmantošana, izvairoties no pārmērīgas atkritumu veidošanās vai attīstot to pārstrādes tehnoloģijas. Aprites ekonomika ir efektīva resursu izmantošana, piemērojot vidi saudzējošus principus, kas papildus citiem ieguvumiem noved pie jaunu uzņēmējdarbības modeļu attīstības un inovatīvu nodarbinātības iespēju radīšanas (MacArthur 2013). Galvenais izaicinājums, lai panāktu pārtikas ražošanas un pārstrādes pieejas pārveidi, ir jaunas izpratnes attīstīšana par vērtīgām pārtikas sastāvdaļām. Ir pilnībā jāpārskata pārtikas atkritumu koncepcija, un šī problēma ir īpaši aktuāla attiecībā uz augļu un ogu pārstrādes atkritumiem. Augļi ir viens no visplašāk patērētajiem pārtikas produktiem, to gada produkcija ir simtiem miljonu tonnu, atkritumu daudzums tikai lielākajās ražotājvalstīs sasniedz desmitiem miljonu tonnu (Wadhwa un Bakshi 2013). Liela daļa šo atkritumu tiek apglabāta atkritumu poligonos, radot apdraudējumu videi. Tajā pašā laikā augļu pārstrādes atkritumi ir materiāls, kas ir bagātīgs ar dažāda veida vielām, piemēram, augļu sēklas satur olbaltumvielas, lipīdus, nukleīnskābes un citas vērtīgas sastāvdaļas, augļu mizas – polisaharīdus, fenolus, alkaloīdus un citas vielas.

Augļu atkritumu pārstrādes problēmu var risināt, izmantojot biorafinēšanu. Biorafinēšana ir ilgtspējīga biomasas pārstrāde bioloģisko produktu (pārtikas, barības, ķīmisko vielu, materiālu) un bioenerģijas ražošanā. Biorafinēšana var nodrošināt vairāku ķīmisko vielu atgūšanu, frakcionējot sākotnējo izejvielu (augļu un augļu atkritumu biomasu) vairākos starpproduktos (ogļhidrātos, olbaltumvielās, taukos un eļļās), ko var tālāk pārvērst produktos ar augstu pievienoto vērtību (Cherubini 2017, Pratima 2013).

Tiek plaši pētīta augļu atkritumu izmantošana, lai izstrādātu jaunus produktus ar funkcionālām īpašībām (funkcionālā pārtika), kas paredzēti lietošanai pārtikā vai citās nozarēs. Ir konstatēts, ka augļu un dārzeņu atkritumu izmantošanas potenciāls ir droša un ekonomiski izdevīga alternatīva sintētiskajām pārtikas piedevām. Biorafinēšanas koncepciju var orientēt uz pievienotās vērtības komponentu ģenerāciju ar daudzveidīgu pielietojuma potenciālu. Atbilstoši šai pieejai biorafinēšanas procesa mērķis ir iegūt maksimāli pilnu to vielu vai vielu grupu spektru, kurām ir pielietojuma potenciāls bioekonomikā. Iespējami lielāka skaita vērtīgu komponentu atgūšana no augļiem un augļu atkritumiem ir pierādījums biorafinēšanas koncepcijas efektivitātei. Viens no galvenajiem faktoriem, kas ietekmē biorafinēšanas metožu izstrādi un biorafinēšanas tehnoloģiju ieviešanu, ir ražošanas procesa ietekmes uz vidi mazināšana.

Savvaļas un kultivētās ogas Latvijas bioekonomikā

Ekoloģiskās, vides, enerģētikas, pārtikas apgādes un dabas resursu problēmas, ar kurām saskaras Eiropa un pasaule, var risināt, izmantojot bioekonomikas principus. Bioekonomika ir "atjaunojamo bioloģisko resursu ražošana un šo resursu un atkritumu plūsmu pārvēršana produktos ar pievienoto vērtību" (EK 2018). Bioekonomika ir noteikta kā viens no Latvijas ilgtspējīgas attīstības stratēģiskajiem mērķiem (Bioekonomikas nacionālā stratēģija 2030). Bioekonomikas izveidei un ieviešanai Latvijā ir liels potenciāls, jo tā var saglabāt un radīt ekonomisko izaugsmi un darba vietas lauku, piekrastes un rūpniecības teritorijās, samazināt atkarību no fosilā kurināmā un uzlabot ekonomisko un vides ilgtspēju. Bioekonomikas panākumu atslēga ir jaunu biorafinēšanas tehnoloģiju izstrāde, lai ilgtspējīgi pārveidotu atjaunojamos dabas resursus bioloģiskos produktos, materiālos un degvielā (2. attēls).



2. attēls. *Vaccinium* ogu spiedpalieku izmantošanas potenciāls.

Vaccinium ģints ogas un to tradicionālā izmantošana

Latvijas purvos un mežos plaši sastopams dabas resurss ir ogas. Ogas tradicionāli izmanto pārtikā un etnomedicīnā. Mūsdienās savvaļas un kultivēto ogu izmantošana pārtikas rūpniecībā, biofarmācijā, kosmētikā gūst arvien lielāku atpazīstamību un uzmanību, jo ir pierādīts ogu lietošanas labvēlīgais efekts uz cilvēku veselību. Ir vairāk nekā 450 sugu, kas pieder pie *Vaccinium ģints* sugām (*Ericaceae* dzimta) (Abreu et al. 2014). Šīs ģints dabiskais areāls ir Ziemeļu puslode, tomēr apmēram 40 sugām ir konstatēta dzimtā vide tropu un subtropu reģionu valstīs (Meksikā, Argentīnā, Gajānā) (Lutein 2007). Daudzas savvaļas un kultivēto ogu sugas, kas saistītas ar labvēlīgu ietekmi uz veselību, pieder pie *Vaccinium* ģints (Nile and Park 2014; Skrovankova et al. 2015). Zināmākie šīs ģints pārstāvji ir mellenes, krūmmellenes, brūklenes un dzērvenes (Abreu et al. 2014). Etnomedicīnā tiek izmantotas 36 *Vaccinium* gints sugas ar vairāk nekā 70 dažādiem pierādītiem izmantošanas efektiem. Šo ogu patēriņš var labvēlīgi ietekmēt gremošanas sistēmu, vielmaiņu, novērst urīnceļu slimības. Plaši tiek izmantotas brūklenes, mellenes un zilenes – šo augu augļus (ogas) izmanto lielākoties pārtikā un tautas medicīnā, savukārt lapas – tikai medicīniskiem mērķiem. Dažādu augu etnobotāniskie lietojumi ir pamats, lai noteiktu kritērijus perspektīviem potenciālo pētījumu virzieniem, kurus varētu veikt, lai atrastu bioaktīvos savienojumus (Abreu et al. 2014).

1.2. *Vaccinium* ogu ķīmiskais sastāvs

Ogu (1. tabula) lietošana uzturā ir ieteikta uztura vadlīnijās visā pasaulē. Neatkarīgi no tā, vai tās ir svaigas, saldētas vai pārstrādātas dažādos produktos, ogu lietošana uzturā nodrošina uzturvielas un fitoķīmiskās vielas veselīgam uzturam (Bazzano 2005). Veselības nozare ir piedāvājusi tirgū dažādus uztura bagātinātājus un uztura sastāvdaļas kā alternatīvu augļu un ogu patēriņam, svaigi produkti bieži vien ir sezonāli, tāpēc aizvien vairāk tiek izmantoti ogu ekstrakti. Ogu lietošanas priekšrocības veselības uzlabošanai ir saistītas ar augstu polifenolu, antioksidantu, minerālvielu, vitamīnu un šķiedrvielu koncentrāciju (Strik 2007).

1. tabula. *Vaccinium* ģints ogu vispārējais sastāvs.

Barības vielas	Mērvienība	Meža mellenes[1]	Lielogu dzērvenes[2]	Krūmmellenes[3]	Purva dzērvenes[4]	Brūklenes[5]	Zilenes[6]
Enerģija	kcal	51	44	57	46	53	52
Mitrums	g	84	83	85	83	82	85
Ogļhidrāti	g	7,1	11,6	10	9,2	11,5	11,8
Olbaltumvielas	g	0,7	0,4	0,7	0,4	0,8	0,6
Tauki	g	0,6	0,1	0,3	0,2	1,2	0,4
Šķiedrvielas	g	5,5	4,4	2,4	4,6	3,7	5,2

[1] Zoratti et al. 2016;[2] McKay un Blumberg 2007;[3] Zorenc et al. 2016;[4] Brown et al. 2012;[5] Bujor et al. 2018;[6] Colak et al. 2016.

Lipīdi ir molekulas, kas ir būtiskas augu un dzīvnieku šūnām. Lipīdi ir atbildīgi par šūnu sieniņu un organoīdu integritāti, tie veido hidrofobu barjeru, kas aizsargā un regulē šūnu funkcijas. Lipīdus var definēt kā primāros metabolītus, jo tie ir būtiski augu augšanai un attīstībai, tomēr dažus lipīdu savienojumus var uzskatīt arī par sekundārajiem metabolītiem, jo tie pilda funkcijas, kas palīdz augiem mijiedarboties ar apkārtējo vidi. *Vaccinium* ģints ogas un to pārstrādes blakusprodukti ir bagāti lipīdu avoti, tie satur taukskābes, sterolus, glicerolipīdus, kuriem kā funkcionāliem savienojumiem ir pozitīva ietekme uz cilvēka veselību. Lai gan lielākā daļa pētījumu par *Vaccinium* ogām koncentrējas uz polifenolu analīzi un bioloģisko aktivitāti, lipīdi joprojām ir svarīga šo ogu sastāvdaļa ar līdzīgu bioloģisko nozīmību.

Polifenoli tiek uzskatīti par vienu no visbagātīgākajām dabisko fitoķīmisko vielu grupām, un tie ir plaši sastopami ogās. Polifenolu grupu pārstāv aptuveni 8000 savienojumi (Leri et al. 2020), kas sastāv no viena aromātiskā gredzena ar vienu vai vairākām hidroksilgrupām, līdz pat polimēriem savienojumiem ar molekulmasu, lielāku par 2500 Da (Rasouli et al. 2017). Galvenās polifenolu grupas ir fenolskābes, stilbēni, flavonoīdi un lignāni.

1.3. *Vaccinium* ģints ogu ekstrakcija un ogu ekstraktu izpēte

Augu izcelsmes fitoķīmisko vielu sagatavošanai, ekstrakcijai un attīrīšanai ir izstrādātas dažādas metodes. Ekstrahējamo vielu veids lielā mērā ir atkarīgs no izmantotās ekstrakcijas metodes, šķīdinātāja un to optimizācijas. Visbiežāk izmantotās ekstrakcijas metodes ir: 1) ekstrakcija ar šķīdinātāju; 2) ekstrakcija ar šķīdinātāju paaugstinātā spiedienā; 3) ekstrakcija ar ultraskaņu; 4) ekstrakcija ar mikroviļņu palīdzību; 5) ekstrakcija ar superkritisko šķidrumu dažādās konfigurācijās. Šīs ekstrakcijas metodes bieži vien papildina ar parastajām šķidruma-šķidruma un šķidruma-cietvielu ekstrakcijas metodēm, lai uzlabotu selektivitāti un neapstrādātā (pirmējā) ekstrakta tīrību (Altemimi et al. 2017). Polifenolu, kā arī lipīdu ekstrakcijā liela nozīme ir ekstrakcijas ilgumam, šķīdinātāja sastāvam, ekstrakcijas intensitātei, lai sagatavotu savienojumam (savienojumu grupai) specifiskas atdalīšanas metodes. Dažādu polifenolu grupu ekstrakcijai visbiežāk izmanto spirta šķīdinātāju sistēmas, kas sastāv no metanola vai etanola (Shi et al. 2005). Heksāns un hloroforms ir ieteicamie šķīdinātāji lipīdu ekstrakcijai, tomēr šo šķīdinātāju toksiskuma un bīstamības dēļ pēdējā laikā priekšroka tiek dota ekstrakcijai ar CO_2 superkritiskā stāvoklī, un, pievienojot šai ekstrakcijas sistēmai polāru vai nepolāru līdzšķīdinātāju, ir iespējams ekstrahēt dažādas polaritātes savienojumus (Lorenzo et al. 2018). Ekstraktu raksturošana tiek veikta, izmantojot spektrofotometriju, dažādus reaģentus, iegūstot specifiskas krāsu reakcijas atkarībā no ekstraktā esošo vielu satura. Kvalitatīvās un kvantitatīvās analīzes, izmantojot hromatogrāfiju ar dažādiem detektoriem (MS, UV, VIS u. c.), kļūst pieejamas, un strauji attīstās jaunu, bioaktīvu dabas vielu identifikācijas stratēģijas.

1.4. *Vaccinium* ģints ogu un to ekstraktu ietekme uz veselību

“Tu esi tas, ko tu ēd” – tā ir labi zināma idioma, kas atspoguļo izpratni par to, cik svarīgs ir veselīgs uzturs un kā tas ir saistīts ar vispārējo labsajūtu. Pastāv pozitīva korelācija starp to, kas tiek uzskatīts par veselīgu uzturu (ko veido augļi, dārzeņi, šķiedrvielas, gaļa ar augstu nepiesātināto tauku saturu u. c.) un veselību uzlabojošu savienojumu koncentrāciju šajos produktos (Berry fruit... 2007, Nile and Park 2014; Bvenura and Sivakumar 2017). Ir labi pierādīta saikne starp polifenolu savienojumu un nepiesātināto taukskābju patēriņu un samazinātu sirds slimību risku, šūnu signalizācijas ceļu regulāciju, tauku transportu, holesterīna sintēzes gaitu. Šo vielu zemais toksiskums padara tās par drošiem uztura elementiem, ar ko uzlabot pārtikas un farmācijas rūpniecības kvalitāti. Kopumā brīvos radikāļus saistošie flavonoīdi un pārtika, kas bagātīga ar šiem sekundārajiem metabolītiem, var palīdzēt cilvēkiem uzturēt veselību, novēršot oksidatīvos bojājumus (Pappas un Schaich 2009, Shaheen un Noreen 2016).

2. MATERIĀLI UN METODES

Izmantotie materiāli un metodes ir detalizēti aprakstītas **1.–13. publikācijā**.

2.1. Augu materiāls

Polifenolu savienojumu ekstrakcijai tika izmantotas 2016.–2020. gada vasaras un rudens sezonā ar rokām ievāktas ogas. Mellenes un brūklenes tika ievāktas Latvijas centrālajā daļā esošās Saulkrastu pilsētas apkārtnes mežos, lielogu dzērvenes un krūmmellenes – Jūrmalas pilsētas nomalē esošajā komercsaimniecībā (Z/S Strēlnieki), purva dzērvenes un zilenes – Ķemeru nacionālajam parkam piederošajos purvos. Sīkāka informācija par ogu izcelsmi un ražas novākšanas laiku atkarībā no attiecīgajiem eksperimentiem atrodama **1.–13. publikācijā**.

Polifenolu ekstrakcijas optimizācija

Polifenolu savienojumu un antociānu ekstrakcijas optimizācija tika veikta, izmantojot lielogu dzērveņu spiedpaliekas. Atbildes virsmas optimizācijā (RSM) tika izmantots etanols un metanols ar trifluoretiķskābes un skudrskābes piedevām (Myers et al. 2016). Tika izmantots divu faktoru un trīs līmeņu centrālais saliktais plāns, kas sastāvēja no vienpadsmit eksperimentiem (trīs atkārtojumi centrālajā punktā). Optimizācijas mainīgie bija etanola/metanola koncentrācija (v/v %) un TFA/skudrskābes koncentrācija (v/v %). Novērotie atbildes mainīgie lielumi bija kopējais antociānu daudzums (mg/100 g ogu materiāla) un kopējais polifenolu daudzums (g/100 g ogu materiāla).

Ekstrakciju no dzērveņu spiedpaliekām tika optimizēta, izmantojot ekstrakciju ar ultraskaņas palīdzību, kā norādīts iepriekšējā pētījumā (**8. publikācija**). Izžāvētu/liofilizētu un homogenizētu ogu vai to spiedpalieku paraugu (0,50 g) nosvēra un pievienoja 50 mL attiecīgā šķīdinātāja maisījuma. Eksperimentos izmantoja 100 W ultraskaņas vannu (Cole-Parmer). Pēc apstrādes ar ultraskaņu paraugus 24 stundas kratīja tumsā un pēc tam filtrēja, lai atdalītu smalkās daļiņas. Tīros, filtrētos ekstraktus uzglabāja tumsā 4 °C temperatūrā.

Lipofīlo vielu ekstrakcija

Kutikulārā vaska ekstrakcijai tika izmantota modificēta metode, izmantojot divus ekstrakcijas šķīdinātājus – hloroformu un heksāna/etilacetāta maisījumu (1:1) (≥ 99%, Sigma-Aldrich, Vācija). Katra ogu suga tika ekstrahēta trīs reizes, izmantojot katru šķīdinātāju. Kopumā katrai ogu sugai tika sagatavoti 6 atkārtojumi. Ekstrakcijai izmantoja trīs 100 mL mērglāzes. Katrā mērglāzē iepildīja 50 mL ekstrakcijas šķīdinātāja. Katram atkārtojumam no ievāktā parauga izvēlējās simts ogas un secīgi citu pēc citas iemērca ekstrakcijas šķīdinātājā uz 30 sekundēm katrā no trijām mērglāzēm, kurās bija šķīdinātājs. Ogu mērcēšanai izmantoja tīras metāla pincetes. Pēc ogu mērcēšanas visu triju mērglāžu saturu filtrēja un apvienoja iztvaicēšanas kolbā. Katru mērglāzi divas reizes mazgāja ar ekstrakcijas šķīdinātāju un pievienoja kombinētajam ekstraktam. Paraugus ietvaicēja pazeminātā spiedienā, izmantojot Rota-Vap ietvaicētāju (Büchi, Vācija). Paraugus ietvaicēja līdz apmēram 5 mL un pārnesa tīrās stikla mēģenēs.

Atlikušo šķīdinātāju ietvaicēja ūdens vannā (40 °C) (Cole Parmer, ASV) zem maigas slāpekļa plūsmas.

Lai ekstrahētu ogu lipīdus, 3 kg ogu tika kaltētas un homogenizētas paraugu smalcinātājā (IKA, Vācija). 120 g homogenizēto ogu nosvēra 1 litra pudelēs ar vāciņu un sajauca ar 600 mL $CHCl_3$, pēc tam uz 20 minūtēm ievietoja ultraskaņas vannā (Cole-Parmer, ASV). Ar ultraskaņu apstrādāto paraugu filtrēja caur papīra filtru. Izmantoto filtrpapīru ar ogu daļiņām ievietoja atpakaļ ekstrakcijas pudelē un pievienoja vēl 600 mL $CHCl_3$. Filtrēšanu un atkārtotu ekstrakciju ar ultraskaņu atkārtoja trīs reizes. Ceturto ekstrakciju veica, inkubējot paraugu $CHCl_3$ uz nakti istabas temperatūrā, lai palielinātu ekstrakcijas iznākumu.

Pēc ekstrakcijas visus ekstraktus filtrēja, apvienoja un koncentrēja, izmantojot rotācijas iztvaicētāju (Heidolph, Vācija). Pēc iztvaicēšanas ogu lipīdus žāvēja slāpekļa plūsmā (AGA, Latvija), sausos paraugus nosvēra un uzglabāja 4 °C temperatūrā.

Ogu vai ogu spiedpalieku ekstrakciju, izmantojot superkritisko CO_2, veica ar augu materiālu, kas bija izžāvēts 40 °C temperatūrā un samalts pulverī (daļiņu izmērs < 1 mm). Ekstrakcijas traukā (tilpums 100 mL) iesvēra 20 g izžāvēta parauga. Ekstrakcijai izmantoja Separex (Francija) superkritiskās CO_2 ekstrakcijas iekārtu, kuras darba parametri bija 250 bāru spiediens, 50 °C temperatūra ar CO_2 plūsmas ātrumu 0,4–0,5 L/min. Tika izmantots līdzšķīdinātājs (70 % etanols). Ekstrakcijas ilgums bija 1 stunda.

Ekstraktu attīrīšana un frakcionēšana

Frakcionēšanai tika izmantoti 1600 mg ogu lipīdu. Nosvērto lipīdu paraugu izšķīdināja heksāna/hloroforma maisījumā. Lipīdu eluēšanu veica pieaugošā eluenta polaritātes secībā: heksāns, heksāns/hloroforms (1:4 v/v), hloroforms, etilacetāts, etilacetāts/metanols (1:1 v/v) un metanols. Frakcionēšanas procesu atkārtoja trīs reizes, savāktās attiecīgā šķīdinātāja frakcijas apvienoja un koncentrēja, izmantojot rotācijas ietvaicētāju, un žāvēja slāpekļa plūsmā.

2.2. Polifenolu analītiskā raksturošana

Antociānu koncentrācija tika noteikta, mērot katra parauga atšķaidījuma absorbciju pie 520 nm un 700 nm, salīdzinot ar demineralizēta ūdens paraugu. Mērījumi tika veikti, izmantojot Shimadzu UV-1800 UV-VIS spektrofotometru. Tika sagatavoti divi buferšķīdumi ar atšķirīgu pH, un paraugus 20–30 minūtes atstāja tumsā, mērījumus veica 20–40 minūšu laikā.

Kopējo polifenolu daudzumu noteica, izmantojot Folēna-Čiokalteu kolorimetrisko metodi (Siriwoharn et al. 2004). Tika sagatavota standarta līkne, izmantojot galluskābi 0–0,350 g/mL robežās (R^2 = 0,999). Mērījumui tika veikti765 nm pēc 20–30 minūšu inkubācijas perioda istabas temperatūrā tumsā. Antociānu identifikāciju un kvantitatīvo noteikšanu veica, izmantojot Waters ACQUITY UPLC sistēmu, kas aprīkota ar šķīdinātāju kontroles sistēmu (QSM), paraugu pārvaldītāju – caurplūdes adatu (SM-FTN) – un fotodiožu bloka (PDA) λ detektoru. Mobilā fāze sastāvēja no 5,0 % skudrskābes un metanola šķīduma ūdenī (70:30 v/v).

2.3. Lipīdu analītiskā raksturošana

Vasku un lipīdu paraugi tika analizēti, izmantojot GC-MS, paraugi tika sagatavoti saskaņā ar iepriekš publicēto metodiku (**1.** un **2. publikācija**). Kopējais atkārtojumu skaits, kas tika veikti ar GC sistēmu, bija 18 katrai analizētajai ogu sugai. Analīzēm tika izmantots GC-2010 gāzu hromatogrāfs, savienots ar GC-MS QP-2010 Ultra masas detektoru (Shimadzu, Japāna). Izmantojamā kolonna bija Restek Rxi®-5MS ar darba temperatūras diapazonu no 40 līdz 350 °C. Ekstrahētos ogu lipīdus un lipīdu frakcijas nosvēra (apmēram 5 mg) GC flakonā un izšķīdināja 1,3 mL piridīna, pievienojot 0,2 mL BSTFA. Pēc tam paraugu 1 h karsēja 60 °C temperatūrā. Iegūto paraugu analizēja, izmantojot GC-MS. Kvantitatīvo noteikšanu veica, vispirms identificējot, pie kuras savienojumu grupas pieder analizējamā viela, un pēc tam aprēķināja koncentrāciju, izmantojot attiecīgo standartu.

2.4. Ekstraktu izmantošanas iespēju novērtējums

Saules aizsardzības faktora (SPF) vērtības tika noteiktas saskaņā ar iepriekš izstrādātu metodi (Mansur et al. 1986). Lipīdu frakciju šķīdumi tika sagatavoti hloroformā koncentrācijā 1,0 mg/mL. Katra šķīduma absorbciju mērīja 290–320 nm diapazonā ik pēc 10 nm trīs reizes, izmantojot Shimadzu UV-1080 UV/VIS spektrofotometru. Pirms mērījumu veikšanas bāzes līniju koriģēja, salīdzinot ar kivetē iepildītu hloroformu.

Ekstrahēto un frakcionēto lipīdu iedarbība tika pārbaudīta uz dažādu veidu mikroorganismiem, izmantojot agara bedrīšu difūzijas metodi. Tika izmantoti mikroorganismi, kas iegūti no Latvijas Mikroorganismu kultūru kolekcijas. Millera-Hintona agara plates inokulēja ar sagatavoto inokulātu. Lipīdu šķīdumi tika sagatavoti DMSO 15 mg/mL koncentrācijā.

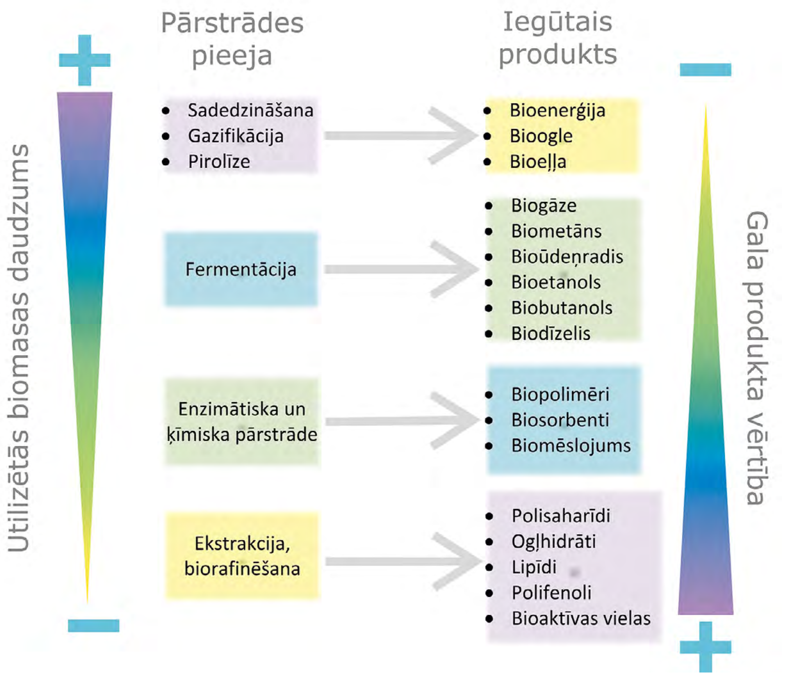
Datu analīze un statistika

Kvantitatīvie kutikulārā vaska sastāva dati tika pakļauti divvirzienu dispersijas analīzei (ANOVA), lai novērtētu atšķirības starp analizētajām ogām, pēc tam tika izmantota Tukeys HSD, lai atšķirtu būtiski atšķirīgās grupas. Lai novērtētu saistību starp dažādām testētajām ogām, tika veikta galveno komponentu analīze (PCA), korelācijas matricas un hierarhiskā klasteru analīze, izmantojot Vorda (*angliski – Ward's*) metodi ar standartizētiem datiem. Statistiskā analīze un datu vizualizācija tika veikta, izmantojot SAS JMP®, 13. versiju (SAS Institute Inc., ASV).

3. REZULTĀTI UN DISKUSIJA

3.1. Ogu spiedpalieku biorafinēšanas stratēģija

Mūsdienu, fosilo resursu izmantošanā bāzētajai ekonomikai pastāv alternatīva – bioekonomika – uz atjaunojamiem resursiem balstīta ražošana, kuras mērķis ir pilnīga biomasas izmantošana. Jaunu produktu ražošana un izstrāde no biomasas balstās biorafinēšanas koncepcijā (Motola et al. 2018). Katru augu biomasas sastāvdaļu var pārstrādāt, ekstrahēt un funkcionalizēt, lai ražotu nepārtikas un pārtikas produktus, starpproduktus tautsaimniecībai (Lange et al. 2021), kā arī enerģiju. Bioloģisko resursu ieguves un pārstrādes blakus plūsmu un atlikumu pārstrāde ir ļoti svarīga, tā ievērojami palielina resursu izmantošanas efektivitāti, tādējādi samazinot oglekļa pēdas nospiedumu, vienlaikus uzlabojot ekonomisko ilgtspēju (EK 2020).



3. attēls. Biomasas atkritumu biorafinēšanas stratēģijas.

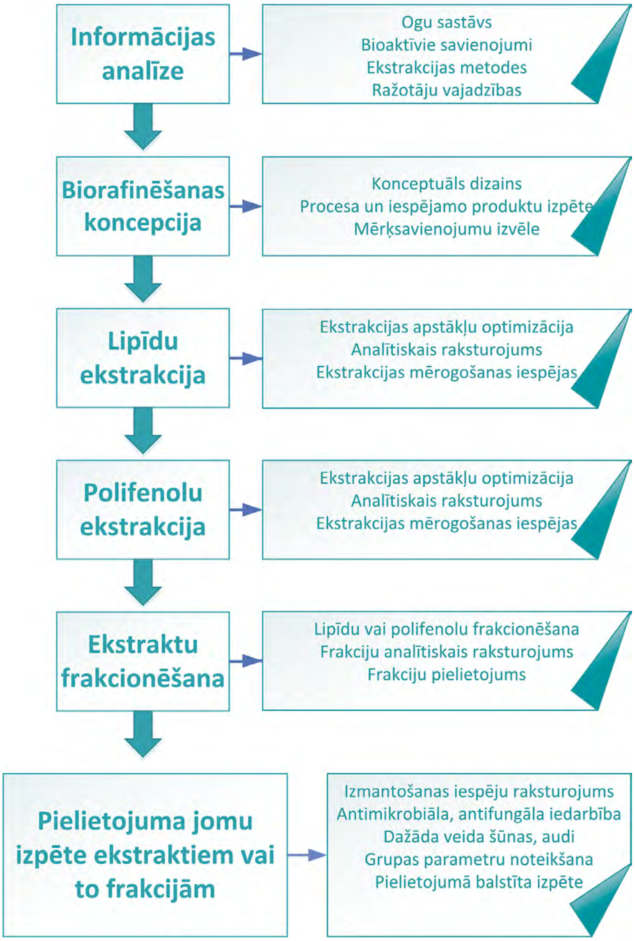
Lai sasniegtu uz bioloģiskiem resursiem balstītas ekonomikas mērķus, galvenā loma ir biorafinēšanas sistēmai, kas atbalsta ļoti efektīvu un rentablu dažādas izcelsmes biomasas pārstrādi izmantojamos produktos, kā arī sekmīgu integrāciju esošajā infrastruktūrā un jau izstrādātajās tehnoloģijās (de Jong et al. 2012, Meyer 2017). Biorafinēšana ir pieeja, kad biomasa tiek secīgi frakcionēta, lai iegūtu produktus ar augstāku vērtību nekā sākotnējā biomasa un/vai enerģiju, tādējādi aizstājot fosilos materiālus (Kamm un Kamm 2004). Var izdalīt trīs galvenās biomasas pārstrādes jomas, kuras nodrošina vielu, materiālu un enerģijas iegūšanu (3. attēls). Par galvenajiem biorafinēšanas produktiem uzskata biodegvielas (bioetanolu, biodīzeļdegvielu, bioogli, bioūdeņradi, metānu un citus), biopolimērus un atsevišķas vielas vai to grupas (pektīnus, fenolus, proteīnus un citus), ko var izmantot tieši vai kas var kalpot kā būvbloki, aizstājot fosilas izcelsmes vielas. Svarīgs mērķis biorafinēšanas metožu attīstībā ir siltumnīcefekta gāzu emisiju samazināšana.

Kā redzams 3. attēlā, dažādas biorafinēšanas stratēģijas ļauj pārstrādāt dažādu veidu un daudzuma biomasas izejvielas, vienlaikus ražojot produktus un enerģiju, kā arī nodrošina iespējas pilnībā aizstāt fosilos materiālus. Dažādās biorafinēšanas stratēģijās būtiski atšķiras biomasas daudzuma pārstrādes iespējas un iegūto produktu pievienotā vērtība.

Biorafinēšanas stratēģijas ir izstrādātas vairākiem biomasas veidiem, bet lielākoties biomasai, kas tiek pārstrādāta lielos apjomos, un kā piemēri minami koksnes biomasas biorafinēšana (Octave un Thomas, 2009), aļģu biomasa (Balina, 2020), apelsīnu spiedpaliekas (Ortiz-Sanchez et al., 2021), lauksaimniecības lignocelulozes biomasa (Clauser et al., 2021) un citas (Nayak un Bhushan, 2019). Ogļhidrāti, lignīns, olbaltumvielas un lipīdi veido apmēram 95 % no augu sastāva pārējie pieci procenti ir vitamīni, polifenoli, krāsvielas, aromātvielas, alkaloīdi vai citas vielas, kurām ir augsta vērtība, ņemot vērā to tiešās izmantošanas potenciālu biorafinēšanas rezultātā. Dažādām augu sastāvdaļām var iezīmēt specifiskas biorafinēšanas iespējas, kuru pamatā ir ogļhidrāti (monosaharīdi, polisaharīdi), lignoceluloze un lipīdi kā galvenie izmantojamu molekulu avoti (Octave and Thomas 2009). Ņemot vērā pārstrādājamās biomasas lielos apjomus, daudzos gadījumos ir pieejamas labi izstrādātas biorafinēšanas stratēģijas (Pauly un Keegstra 2008, Chen un Dixon 2007).

Īpaša biomasas atkritumu grupa ir pārtikas atkritumi, jo tie rodas pārtikas produktu pārstrādes procesā un ir bagātīgi ar vielām, kam ir augsta uzturvērtība un kas arī satur citas vielas ar, iespējams, augstu izmantošanas potenciālu (Nayak and Bhushan 2019), piemēram, ogu sulas ražošanas rezultātā radušās ogu spiedpaliekas (izspiednes). Līdz šim ogu, īpaši *Vaccinium* ģints ogu, spiedpalieku biomasas biorafinēšanas stratēģijas nav padziļināti izstrādātas un izmantotas. *Vaccinium* ģints ogu spiedpalieku biorafinēšanas stratēģijas izstrādes īpaši aspekti ir:

1. Salīdzinoši neliels biomasas (*Vaccinium* ogu spiedpalieku) apjoms, kas ir perspektīvs biorafinēšanai. Sulas ražošanai no *Vaccinium* ogām visplašāk tiek izmantotas lielogu dzērvenes. 2019. gadā pasaulē, galvenokārt ASV, Kanādā un Čīlē, saražots 687 535 tonnu lielogu dzērveņu, Eiropā – ievērojami mazāk. Latvijā 2019. gadā saražots 344 tonnu lielogu dzērveņu (World cranberry production ... 2019). Ogu sulas ražošana parasti ir decentralizēta, tādējādi arī ogu atkritumu pārstrādes apjoms (biorafinēšana) varētu būt salīdzinoši neliels, mazāks nekā jebkurš process, kas vērsts, piemēram, uz lignocelulozes vai aļģu biomasas pārstrādi.
2. Biorafinēšanas procesa rezultātā iegūto vielu grupu vai atsevišķu vielu tirgus ir atkarīgs no izmantošanas iespējām. Ogu spiedpalieku pārstrādes produktu izmantošanas perspektīvas nosaka to sastāvā ietilpstošas vielas ar augstu bioloģisko un farmakoloģisko aktivitāti, piemēram, polifenoli (vielu grupas, piemēram, antocianīni, procianidīni) vai atsevišķas vielas, piemēram, resveratrols. Cita izmantošanai nozīmīga vielu



4. attēls. *Vaccinium* ogu spiedpalieku biorafinēšanas plāns.

grupa, ko iespējams izolēt no ogu spiedpaliekām, ir lipīdi (vaski, taukskābes, sterīni, terpēni u. c.) un ēteriskās eļļas. Tādējādi biorafinēšanas stratēģijai būtu jākoncentrējas uz vērtīgāko komponentu pārstrādi, jo pēc polifenolu un lipīdu izdalīšanas atlikumu vērtība ir ievērojami zemāka un to pārstrāde dārgāka, atbalstot iespējas izmantot utilizācijas tehnoloģijas, piemēram, kompostēšanu vai pēc ekstrakcijas radušos atkritumu integrēšanu citu biomasas plūsmu pārstrādes tehnoloģijās.

3. *Vaccinium* ogu spiedpalieku kvalitāte salīdzinājumā ar citiem pārtikas pārstrādes atkritumiem ir augsta, jo tās satur vairāk bioaktīvu savienojumu. Pārtikas atkritumu kvalitāte ir viena no galvenajām problēmām saistībā ar to biorafinēšanu, jo pēc pārstrādes fermentatīvās hidrolīzes rezultātā ļoti ātri var sākties to degradācija, veidoties piesārņojums ar mikroorganismiem. *Vaccinium* ogu spiedpalieku pārstrādes ciklā ogas pirms sulas ražošanas tiek attīrītas, un pēc sulas iegūšanas tās ir iespējams izžāvēt vai sasaldēt, lai novērstu kvalitātes pasliktināšanos. Tādējādi bioloģiski aktīvās sastāvdaļas paliek neskartas.

4. Zināšanu līmenis par ogu spiedpalieku, īpaši *Vaccinium* ogu, sastāvu un iespējamām biorafinēšanas pieejām ir nepilnīgs. Neraugoties uz nesenu pētījumu rezultātiem par *Vaccinium* ogu sastāvu un nedaudzajiem pētījumiem, kas saistīti ar *Vaccinium* ogu spiedpalieku sastāva izpēti, trūkst zināšanu, kas nepieciešamas, lai izstrādātu ogu spiedpalieku biorafinēšanas stratēģiju. Ir izstrādāti daži priekšlikumi dzērveņu spiedpalieku biorafinēšanai (Harrison et al. 2013). Būtisks esošo pētījumu trūkums ir toksisku un bīstamu šķīdinātāju izmantošana ogu pārstrādē. Līdz ar to ir svarīgi attīstīt videi draudzīgas ekstrakcijas metodes, kā arī optimizēt ekstrakcijas metodes, kas nepieciešams izmēģinājuma mēroga vai rūpnieciskās ražošanas procesu izstrādei.

Vaccinium ogu spiedpalieku biorafinēšanas plāna izstrādes mērķis ir paplašināt zināšanas par šo ogu vērtīgajām sastāvdaļām, kā arī izveidot efektīvu un modulāru biorafinēšanas pieeju, lai gūtu maksimālu labumu no šā vērtīgā biomasas veida (4. attēls).

3.2. Ogu un to spiedpalieku lipīdi

Viena no galvenajām vielu grupām ogās un to spiedpaliekās ir lipīdi. Pētījumi par ogu lipīdiem ietver paraugu sagatavošanu, ekstrakciju, analītisko raksturojumu un iespējamo pielietojuma jomu izpēti. Tika izvirzīti vairāki uzdevumi veselu ogu un to spiedpalieku lipīdu starpdisciplinārai izpētei, lai attīstītu izmantošanas risinājumus, tādējādi veicinot bioekonomikas attīstību.

3.2.1. Ogu lipīdu ekstrakcijas optimizācija

Lipīdi ir funkcionāli savienojumi, kas ir būtiski jebkuram dzīvajam organismam. Lipīdus uzskata par primārajiem metabolītiem, jo bez tiem organisma darbība nebūtu iespējama. Lipīdi pilda dažādas bioloģiskās funkcijas, un to klasifikācija ir sarežģīta – dažas klasifikācijas sistēmas ir balstītas uz lipīdu funkcijām, citas – uz strukturālajām līdzībām. Cits aspekts savienojumu klases definēšanā ir daudz vienkāršāks – lipīdi nešķīst ūdenī, bet šķīst organiskajos šķīdinātājos. Ogu lipīdu izpēte ir balstīta uz pēdējo minēto definīciju apvienojumā ar jaunāko un visaptverošāko klasifikācijas sistēmu, ko piedāvā LIPID-MAPS (Liebisch et al. 2020).

Ogas nosacīti veido 3 galvenie komponenti – sēklas, miza un mīkstums. Kopumā dažādu ogu sēklas sastāv no lipīdiem, kas tiek izmantoti enerģijas uzkrāšanai: taukskābēm, glicerolipīdiem un lipīdiem, kuri piedalās šūnas funkcionēšanas regulācijā. Mizas sastāv no steroliem (augu hormoni, attīstības un augšanas regulēšana, transmembrānu caurlaidības regulēšana, adaptogēni) un prenollipīdiem (nodrošina dažādas funkcijas šūnās, darbojas kā aizsargkomponenti pret patogēniem), kā arī vaskiem (sīkāk aplūkoti promocijas darba 3.3. nodaļā) (garas ķēdes alifātisko ogļūdeņražu, alkānu, esteru, garas ķēdes taukskābju, ketonu, aldehīdu, primāro un sekundāro spirtu maisījums, kas aizsargā augus no biotiskā un abiotiskā stresa) (Bederska-Łojewsk et al. 2021). Mīkstumā ir ir dažādas taukskābes (dažāda garuma, līdz C22), sterīni, triterpenoīdi un citi mazāk nozīmīgi lipīdi, augsta ogļhidrātu un polifenolu koncentrācija (Tundis et al. 2021). Ņemot vērā iespējamās savienojumu grupas ogās un to daļās, attiecīgi jāizvēlas ekstrakcijas šķīdinātāji un metodes. Tā kā savienojumiem ir atšķirīga polaritāte, tika pārbaudīti dažādi ekstrakcijas šķīdinātāji, lai optimizētu lipīdu ekstrakciju, kas nodrošina augstāko iznākumu.

Ir konstatēts, ka *Vaccinium* ogās ir no 6,90 g līdz 9,17 g lipīdu/100 g žāvētu ogu (2. tabula). Tomēr šie rezultāti lielā mērā ir atkarīgi no izmantotās ekstrakcijas metodes un parametriem, piemēram, šķīdinātāja un ogu masas attiecība, ekstrakcijai lietotās ultraskaņas jaudas, ekstrakcijas temperatūras, gatavojamā ekstrakta kopējā tilpuma un citiem faktoriem. Turpmāki pētījumi (**3. publikācija**) liecina: lai iegūtu preparatīvus ogu lipīdu daudzumus, nepieciešams lielāks ogu biomasas daudzums un lielāks šķīdinātāja tilpums. Vēl viens aspekts, kas attiecas uz dažādos pētījumos (**1., 2.** un **3. publikācija**) norādītajiem atšķirīgajiem ieguvumiem, ir ogu kvalitāte, jo īpaši ogu novākšanas laiks, ogu gatavība, paraugu sagatavošana (ogu žāvēšana, malšana).

2. tabula. Lipīdu ekstrakcijas iznākums no dažādām ogām, kā ekstrakcijas šķīdinātāju izmantojot hloroformu.

Ogas	Ekstrakcijas iznākums, g ekstrakta/100 g svaigu ogu
Lielogu dzērvenes	9,17
Meža mellenes	8,37
Brūklenes	9,05
Purva dzērvenes	7,57
Zilenes	8,66
Krūmmellenes ‘Blue crop’	6,90
Krūmmellenes ‘Blue gold’	7,84
Krūmmellenes ‘Blue ray’	7,10
Krūmmellenes ‘Chippewa’	7,51
Krūmmellenes ‘Duke’	8,15
Krūmmellenes ‘North blue’	8,18
Krūmmellenes ‘Patriot’	7,65
Krūmmellenes ‘Polaris’	7,46

Kā videi draudzīga ekstrakcijas metode ar potenciālu pielietojumu rūpniecībā tika pārbaudīta un novērtēta ekstrakcija, izmantojot CO_2 superkritiskā stāvolī (SCO_2), pamatojoties uz ekstrakcijas iznākumu (3. tabula). Tika ekstrahētas veselas izžāvētas ogas un maltas ogu spiedpaliekas, izmantojot etanolu kā līdzšķīdinātāju (lai nodrošinātu iegūtā ekstrakta plūsmu sistēmā). Iegūtajiem ekstraktiem ir līdzīgs sastāvs kā ekstraktiem, kas iegūti, izmantojot nepolāros šķīdinātājus (hloroformu, heksānu, petrolēteri, etilacetātu), nodrošinot salīdzināmu ekstrakcijas iznākumu. Salīdzinājumā ar ekstrakciju ar šķīdinātājiem, kur šķīdinātāju var atgūt un atkārtoti izmantot turpmākai ekstrakcijai, SCO_2 ekstrakcijas laikā CO_2 atkārtoti cirkulē caur sistēmu, turklāt CO_2 iztvaiko no ekstrakta, neatstājot ekstraktos kaitīgas atliekas. Ņemot vērā šīs priekšrocības, SCO_2 ekstrakcijas iekārtu attīstību un pieaugošo pieejamību arī rūpnieciskā mērogā, šī pieeja ir kļuvusi par dzīvotspējīgu variantu lipīdu ekstrakcijai no dažādiem materiāliem, kuru raksturo pierādīta efektivitāte, zemas oglekļa dioksīda emisijas un samazinātas produktu izmaksas, izmantojot šāda veida ekstrakciju.

3. tabula. Lipīdu ekstrakcijas iznākums no *Vaccinium* ogām, kā ekstrakcijas šķīdinātāju izmantojot oglekļa dioksīdu superkritiskā stāvoklī.

Ogas	Materiāla veids	Ekstrakcijas iznākums, g/100 g ogu
Lielogu dzērvenes	Veselas ogas	8,71
	Spiedpaliekas	14,48
Meža mellenes	Veselas ogas	822
	Spiedpaliekas	10,53
Krūmmellenes	Veselas ogas	7,18
	Spiedpaliekas	10,45
Brūklenes	Veselas ogas	8,87
	Spiedpaliekas	11,48

3.2.2. Iegūto lipīdu ekstraktu kvalitatīvā un kvantitatīvā analīze

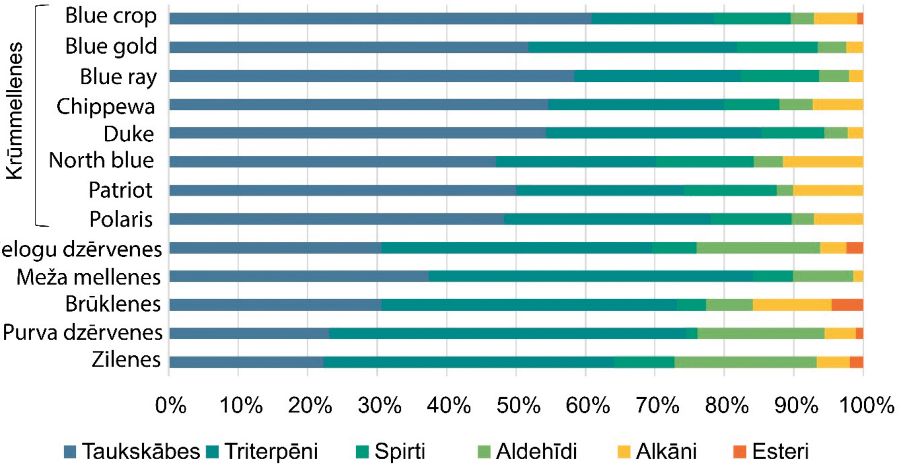
Iegūtie lipīdu ekstrakti tika analizēti, izmantojot GC/MS, lai noteiktu to kvalitatīvo un kvantitatīvo sastāvu. Kvalitatīvās un kvantitatīvās analīzes galvenais mērķis bija noteikt dažādu ogu lipīdu ekstraktos esošās vielas, pamatojoties uz izmantoto materiālu (veselas, svaigas ogas, ogu spiedpaliekas), un tādējādi atbalstīt jaunu bioekonomikas lietojumu izstrādi.

Tika novērtēta šķīdinātāja ietekme uz atsevišķu vielu sastāvu un ekstrakcijas efektivitāti (**1. publikācija**). Tika kvantitatīvi noteiktas un salīdzinātas 22 atsevišķas vielas, kas bija sastopamas visos sagatavotajos ekstraktos, pamatojoties uz izmantoto šķīdinātāju. Kvantitatīvi noteikto vielu kopējais ekstrakcijas iznākums bija vislielākais, ja tika izmantots dietilēteris, vismazāk efektīvs bija petrolēteris.

Identificētie ogu lipīdu savienojumi tika iedalīti 6 lipīdu apakšklasēs. Lipīdu apakšklase, kas tika atrasta augstākajā koncentrācijā, ir taukskābes (līdz 60 % no kopējā lipīdu daudzuma krūmmelleņu šķirnēs). Galvenās taukskābes, kas konstatētas pētītajās ogās, ir palmitīnskābe, linolskābe un linolēnskābe (5. attēls).

Vielu grupa, kas bieži sastopama lipīdu ekstraktu sastāvā, ir alkāni. Šie savienojumi ir ogu epikutikulārā vaska sastāvdaļa, un ogu citoplazmā tie ir augu metabolītu biosintēzes prekursori. Alkānu oglekļa ķēdes garums ogās ir no C19 līdz C31. Kā nozīmīgākais no alkāniem ogās tika konstatēts C29 alkāns (nonakozāns). No pētītajām savvaļas ogām vislielākā nonakozāna koncentrācija tika konstatēta brūklenēs (6,81 g/100 g). Savvaļas mellenēm un zilenēm tika novērota zema alkānu koncentrācija, mellenēs tika konstatēti tikai C25 un C29 alkāni. Pētītajos krūmmelleņu šķirnēs alkānu saturs bija daudz plašāks, piemēram, melleņu šķirnē 'North blue' tika atrasti 8 dažādi alkāni, no kuriem augstākajā koncentrācijā bija nonakozāns.

Lipīdu ekstraktu kopējā daudzuma vispusīga analīze ir būtisks solis, lai noteiktu lipīdu ekstraktu izmantošanas jomas. Turklāt frakcionēšana ļauj gūt skaidrāku



5. attēls. Identificēto lipīdu savienojumu grupu relatīvais daudzums pētītajās *Vaccinium* ogās.

priekšstatu par pielietojuma jomām, jo vieglāk var identificēt vielas vai to grupas, kas atbildīgas par konkrētām aktivitātēm, frakcionēšana ļauj sagatavot tīrākas frakcijas, kurām būs lielāks pielietojuma potenciāls. Sīki izstrādātu lipīdu ekstraktu no *Vaccinium* un citu sugu ogām, kā arī to šķirņu sīkāka ķīmiskā analīze atrodama **1.**, **2.** un **3. publikācijā**.

3.2.3. Lipīdu ekstraktu pielietojuma identifikācija

Ogu lipīdu saules aizsardzības faktors

Dažādiem augu ekstraktiem ir izteiktas spējas aizkavēt Saules starojumu (saules aizsardzības faktors; SPF), un tie tiek izmantoti kā UV filtri saules aizsarglīdzekļos, tomēr ogu lipīdu izmantošana šādiem mērķiem līdz šim nav izvērtēta (Sutar et al. 2020). Iegūtās SPF vērtības kopējiem ekstraktiem bija 3,6 un 9,4 attiecīgi mellenēm un brūklenēm. Brūkleņu kopējais ekstrakts saturēja lielu daudzumu fenolskābju un izoprenoīdu, kuru nebija melleņu ekstraktā. Kopumā pārbaudīto frakciju SPF vērtības palielinās, palielinoties izmantotā eluēšanas šķīdinātāja polaritātei, un sasniedz maksimālās SPF vērtības, kad kā eluentu izmanto etilacetātu/metanolu. Visaugstākās SPF vērtības tika iegūtas etilacetāta/metanola frakcijai: 11,6 – mellenēm, 10,2 – brūklenēm. Melleņu eilacetāta/metanola frakcija satur lielu daudzumu taukskābju, proti, C18 nepiesātināto taukskābju (157 mg/g ekstrakta), un kanēļskābes (27 mg/g), brūkleņu frakcija satur 27 mg/g un 11 mg/g attiecīgo savienojumu, abās ogu frakcijās ir arī liels benzoskābes saturs (36 un 197 mg/g). Iegūtās SPF vērtības liecina, ka ogu lipīdi ir potenciāli izmantojami saules aizsarglīdzekļu ražošanā, tādējādi aizstājot sintētiskās un neorganiskās saules aizsarglīdzekļu sastāvdaļas ar dabiskām un ilgtspējīgām sastāvdaļām ar mazu ietekmi uz vidi, vienlaikus veicinot bioekonomiku un pārtikas atkritumu valorizāciju.

Ogu lipīdu frakciju antimikrobiālā aktivitāte

Kopējo ogu lipīdu ekstraktu un ekstraktu frakciju antimikrobiālā aktivitāte tika pārbaudīta pret sešiem cilvēku patogēniem vai oportūnistiskiem patogēniem, izmantojot agara iedobes difūzijas metodi. Iegūtās frakcijas uzrādīja antimikrobiālu aktivitāti pret *S.aureus, S.pyogenes, S.epidermidis* un *E.coli,* bet netika konstatēta aktivitāte pret *P.mirabilis* un *P.aeruginosa.* Melleņu kopējais ekstrakts un tā frakcijas uzrādīja augstu inhibīcijas potenciālu, testējot ar *S.aureus*. Hloroforma un etilacetāta frakcija uzrādīja ievērojamu antimikrobiālo aktivitāti, šīs frakcijas bija īpaši bagātīgas ar β-sitosterolu. Šīm frakcijām bija inhibējoša iedarbība pret citām grampozitīvajām baktērijām *S.pyogenes* un *S.epidermidis*. *S.pyogenes* inhibēja visas pārbaudītās melleņu frakcijas. Kopējā lipīdu ekstrakta inhibīcija ir līdzīga sagatavoto frakciju inhibīcijai, kas norāda uz iespējamu ogu lipīdu sinerģisku iedarbību, kura samazina mikrobu augšanu. Tā kā pārtikas rūpniecība un ogu pārstrāde pasaulē pastāvīgi pieaug, ogu lipīdiem, ko varētu iegūt no ogu spiedpaliekām, var būt nozīmīga loma jaunu, lietotājam draudzīgu, inovatīvu produktu izstrādē.

3.3. Ogu un to spiedpalieku vaski

Ogu un augu vaski kopumā sastāv no garas ķēdes alifātisko ogļūdeņražu, alkānu, esteru, ēteru, garas ķēdes taukskābju, ketonu, aldehīdu, primāro un sekundāro spirtu maisījuma, kas aizsargā augus no biotiskā un abiotiskā stresa. Ogu un to spiedpalieku vaska pētījumu mērķis bija izprast ogu kutikulārā vaska funkcijas kā augu aizsardzības sistēmas sastāvdaļu, parādīt ogu vaska praktiskā pielietojuma iespējas, ņemot vērā vasku kā vienu no funkcionālajiem komponentiem, kas iegūts biorafinēšanas procesā.

3.3.1. Ogu vaska ekstrakcija

Promocijas darbā tika pētīts ogu kutikulārā vaska slānis, kas sastāv no intrakutikulārā vaska (amorfie lipīdi, kas iestrādāti kutīnā) un ārējā epikutikulārā vaska (Jetter et al. 2008). Tā kā nav iespējams atdalīt abus vaska slāņus, kas atrodas ogu ārējā daļā, abi šie slāņi jāiegūst vienlaikus. Lai izolētu tieši ogu kutikulārā vaska slāni, jāizmanto svaigas ogas. Ogu spiedpaliekas satur ogu daļu maisījumu, tāpēc šāda materiāla kutikulārā vaska sastāva iegūšana un noteikšana nav iespējama. Tomēr ogu spiedpalieku vasku var iegūt kā produktu, izmantojot SCO_2 ekstrakciju, kas dod iespēju iegūt ogu vaskus rūpnieciskā mērogā.

Dažādu ogu sugu kutikulārā vaska ekstrakcija tika veikta, izmantojot hloroformu, kā ieteikts iepriekš (Jetter et al. 2008). Tika pētītas šādas ogas: zilenes (*Vaccinium uliginosum* L.), meža mellenes (*Vaccinium myrtillus* L.), lielogu dzērvenes (*Vaccinium macrocarpon*), brūklenes (*Vaccinium vitis-idaea* L.), melnās vistenes (*Empetrum nigrum* L.), goltjēras (*Gaultheria mucronata),* pīlādži (*Sorbus aucuparia* L.), vilkābele (*Crataegus alemanniensis)* un astoņas krūmmelleņu (*Vaccinium corymbosum* L.) šķirnes, proti, 'Blue crop', 'Blue gold', 'Chandler', 'Chippewa', 'Duke', 'North blue', 'Patriot' un 'Polaris'. Iegūtie ekstrakcijas rezultāti (4. tabula) liecina, ka ogās, kurām ir glancēts, gluds kutikulārā vaska slānis (neveidojas vaska kristāli), piemēram, brūklenēs, purva dzērvenēs un lielogu dzērvenēs, ir lielāka kutikulārā vaska koncentrācija nekā ogās, kurām ir matēts, strukturēts kutikulārā vaska slānis (veidojas kristāli), piemēram, mellenēs un krūmmellenēs.

4. tabula. Pētītās ogas, to taksonomiskā saistība pēc dzimtas, sugas un vaska daudzuma uz vienas ogas. ± ir vaska daudzuma standartnovirze (*n = 3*).

Pētītās ogas	Dzimta	Suga	Šķirne	Vasks, mg/oga
Vilkābele	Rosaceae	*Crataegus*		1,43 ± 0,09
Pīlādzis	Rosaceae	*Sorbus*		1,48 ± 0,09
Goltjēra	Ericaceae	*Gaultheria*		0,65 ± 0,02
Melnā vistene	Ericaceae	*Empetrum*		1,71 ± 0,11
Zilene	Ericaceae	*Vaccinium*		0,95 ± 0,09
Meža mellene	Ericaceae	*Vaccinium*		0,63 ± 0,05
Brūklene	Ericaceae	*Vaccinium*		1,89 ±0,09
Lielogu dzērvene	Ericaceae	*Vaccinium*		1,46 ± 0,12
Krūmmellenes	Ericaceae	*Vaccinium*	‘Blue crop’	0,74 ± 0,04
			‘Blue gold’	0,67 ± 0,03
			‘Chandler’	0,83 ± 0,05
			‘Chippewa’	0,90 ± 0,07
			‘Duke’	0,57 ± 0,02
			‘North blue’	0,65 ± 0,02
			‘Patriot’	0,84 ± 0,03
			‘Polaris’	0,87 ± 0,03

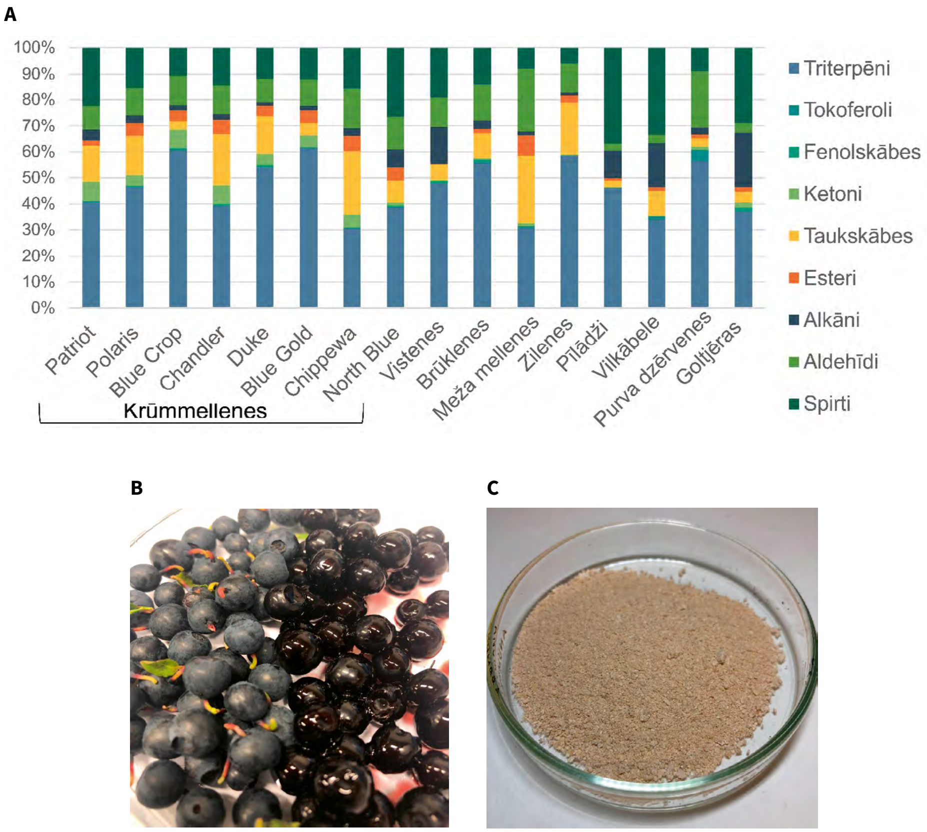
Tiek uzskatīts, ka ģeogrāfiskā izcelsme, respektīvi, vieta, kurā ogas ievāktas, ietekmē ogu ķīmisko sastāvu, tostarp vaska sastāvu un daudzumu uz ogu virsmas. Šī hipotēze tika pārbaudīta, vācot mellenes un ekstrahējot to kutikulāro vasku no ogām Latvijā, Norvēģijā un Somijā divās secīgās vasaras sezonās (2018. un 2019. gadā). Iegūtie rezultāti parādīja, ka, pirmkārt, atkarībā no ražas gada var būtiski mainīties vaska blīvums uz ogas virsmas (mg vaska uz ogu), otrkārt, vaska daudzums mainās atkarībā no platuma grādiem, kuros mellenes ievāktas (**7. publikācija**).

SCO_2 ekstrakciju veica, izmantojot izžāvētas samaltas ogu spiedpaliekas, un preparatīvos daudzumos tika iegūts vaskains, sauss materiāls. Tā kā ogu spiedpaliekas satur citus ogu komponentus, tostarp sēklas, iegūtie ekstrakcijas daudzumi bija lielāki, un arī vasks bija atšķirīgs, jo, izmantojot šādu ekstrakcijas metodi, nav iespējams nodrošināt selektivitāti. Ekstrakti, kas tika sagatavoti, izmantojot SCO_2 ekstrakciju, saturēja 1,02 % un 0,45 % vasku attiecīgi brūkleņu un melleņu spiedpaliekās. SCO_2 ekstrakcija parādīja iespēju iegūt vasku no sulas ražošanas atkritumiem to perspektīvai izmantošanai pārtikas vai kosmētikas rūpniecībā.

3.3.2. Vaska ekstraktu kvalitatīvā un kvantitatīvā analīze

Ogu vaski satur deviņas savienojumu grupas – alkānus, fitosterolus, triterpenoīdus, spirtus, taukskābes, fenolskābes, ketonus, tokoferolus un aldehīdus. Atkarībā no ogas sugas un tās kutikulārā vaska morfoloģijas sastāvs var atšķirties, un galvenās savienojumu grupas var atšķirties ne tikai starp sugām, bet arī starp vienas sugas šķirnēm (6. attēls). Fenolskābes un tokoferoli tika atklāti kā maznozīmīgas ogu vaska sastāvdaļas; neraugoties uz to salīdzinoši nelielo daudzumu, tiem ir būtiska nozīme augu un patogēnu mijiedarbībā. Saskaņā ar citām publikācijām fenolskābes un tokoferoli var attiecīgi aizsargāt pret UV starojumu un veicināt antibakteriālo aktivitāti (**4.** un **5. publikācija**). Triterpenoīdi, taukskābes un spirti ir trīs galvenās kutikulārā vaska savienojumu klases, kas veido attiecīgi līdz 62 %, 31 % un 38 % no kopējā vaska satura dažādās krūmmelleņu šķirnēs. Ķīmisko savienojumu klašu sastāvs un sadalījums var atšķirties atkarībā no izcelsmes vietas un ģenētiskā fona (**6.** un **7. publikācija**).

Tika pētīts melleņu kutikulārā vaska sastāvs dažādās to attīstības stadijās. Līdzīgi **5. publikācijā** sniegtajiem rezultātiem arī savvaļas tipa un līdzena vaska pārklājuma



6. attēls. Noteikto savienojumu grupu relatīvais daudzums dažādu ogu vaskos (A); pa kreisi – novāktās meža mellenes ar redzamu matētu vaska slāni, pa labi – ar hloroformu ekstrahētās mellenes, kurām vaska slānis ir ekstrahēts (B); meža melleņu kutikulārais vasks, kas ekstrahēts, izmantojot mērcēšanas metodi ar hloroformu (C).

melleņu kutikulāro vasku kā galvenās savienojumu klases veidoja galvenokārt triterpenoīdi un taukskābes. Ogās ar līdzenu vaska pārklājumu bija mazāks taukskābju daudzums un lielāks triterpenoīdu daudzums visos attīstības posmos nekā savvaļas tipa ogās. Abos ogu tipos vēlākajos nogatavošanās posmos kutikulārajā vaskā palielinājās alkānu daudzums (**6. publikācija**).

SCO_2 ekstrakcija no melleņu un brūkleņu spiedpaliekām liecina par iespējām ekstrahēt ogu vaskus, mainot ekstrakcijas parametrus. SCO_2 ekstraktos galvenās taukskābes bija linolskābe un γ-linolēnskābe, kas veidoja aptuveni 50 % no kopējā vasku daudzuma (**4. publikācija**).

Vaska ekstraktu un to sastāvdaļu kvalitatīvā un kvantitatīvā analīze liecina, ka vaska sastāvs lielā mērā ir atkarīgs no attiecīgo ogu ģenētiskās izcelsmes, izcelsmes vietas un izmantotās ekstrakcijas metodes. Ekstrakcija, izmantojot hloroformu (ogu mērcēšana), īpaši izolē ārējo vaska slāni, savukārt SCO_2 ekstrakcija ir intensīvāka, jo īpaši attiecībā uz ogu spiedpaliekām, kas ir visu ogu nodalījumu maisījums.

3.3.3. Vaska ekstraktu pielietojuma identifikācija

Ogu vasku saules aizsardzības faktora (SPF) noteikšana

Saules aizsardzības faktors ir vērtība, ko nosaka produktiem, kuri spēj bloķēt UV-B starus, kas var nelabvēlīgi ietekmēt cilvēka ādu. Komerciāli pieejamos saules aizsarglīdzekļos papildus vairākām sintētiskām sastāvdaļām parasti izmanto nanoizmēra ZnO (efektīvāk aizsargā pret UV-A starojumu) vai TiO_2 (efektīvāk aizsargā pret UV-B starojumu). Ņemot vērā negatīvo ietekmi uz vidi un cilvēku veselību, būtu jāapsver videi un cilvēka veselībai draudzīgi UV aizsardzības risinājumi. Viens no šādiem risinājumiem varētu būt ogu lipīdu (3.2.3. sadaļa) vai vaska kā pārtikas atkritumu biorafinēšanas produkta izmantošana.

Iegūtie rezultāti attiecībā uz ogu vasku un ogu spiedpalieku vasku liecina par SPF vērtību palielināšanos atkarībā no devas (5. tabula). Melleņu un brūkleņu spiedpalieku vasks, kas ekstrahēts, izmantojot SCO_2, uzrāda augstākas SPF vērtības nekā tie vaski, kas ekstrahēti, izmantojot hloroformu. Sastāva analīze parādīja, ka spiedpalieku vaska ekstrakti satur lielāku daudzumu nepiesātināto taukskābju, kas varētu noteikt augstāku SPF vērtību (**4. publikācija**).

5. tabula. Ogu vaska SPF vērtības dažādām vaska koncentrācijām, kas iegūtas, izmantojot hloroformu, un no veselām ogām un spiedpaliekām, izmantojot SCO_2.

	1 mg/mL	**2 mg/mL**
Ogu vasks (hloroforma ekstrakts)		
Meža mellenes	4,5 ± 0,9	7,5 ± 1,8
Brūklenes	4,8 ± 1,5	12,7 ± 0,8
Zilenes	14,8 ± 1,3	25,4 ± 0,2
Ogu spiedpalieku ekstrakts (SCO_2)		
Meža mellenes	7,9 ± 0,3	15,09 ± 0,12
Brūklenes	6,1 ± 0,5	13,4 ± 0,9

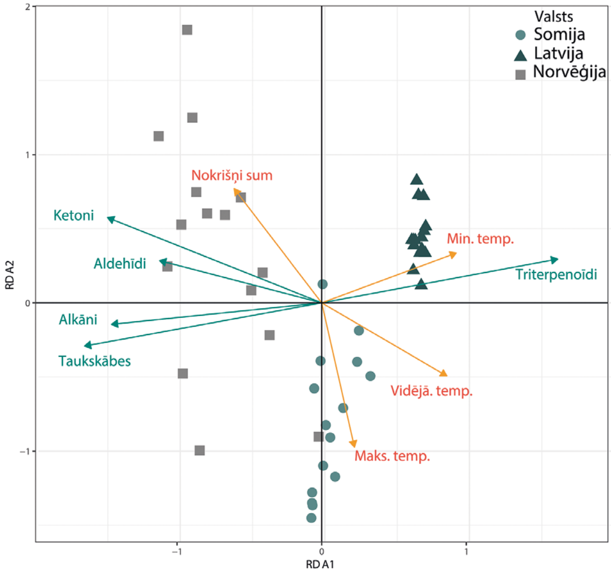
[*] ± ir mērījumu SD (*n* = 3).

Autentiskuma pārbaude, izmantojot kvantitatīvus ogu vaska sastāva datus

Galveno komponentu analīzes (PCA) diagrammas parāda, ka novērotās atšķirības ogu vasku sastāvā var izskaidrot ar atšķirībām dažādu grupu ķīmisko vielu koncentrācijā. Datus par krūmmelleņu šķirnēm var iedalīt atsevišķās grupās, ja tos attēlo, grupējot pēc taukskābju sastāva. Katrai analizētajai krūmmelleņu šķirnei ir unikāls taukskābju sastāvs. Tas liecina, ka taukskābes un to variācijas melleņu kutikulārajā vaskā var izmantot kā instrumentu dažādu šķirņu atšķiršanai. Kā parādīts **5. publikācijā,** ogu vaska sastāva analīzi var veiksmīgi izmantot, lai atšķirtu dažādas ogu sugas un pat vienas sugas dažādas šķirnes.

Klimata apstākļi ietekmē ogu vaska sastāvu un saturu

Triterpenoīdi, taukskābes, alkāni, aldehīdi, ketoni un pirmējie spirti bija galvenie savienojumi, kas tika atklāti meža melleņu vaskā, kuru paraugi tika ievākti Norvēģijā, Latvijā un Somijā (7. attēls). Ģeogrāfiski atšķirīgajās paraugu ievākšanas vietās un dažādos gados melleņu kutikulārajā vaskā bija vērojamas vaska komponentu koncentrācijas un relatīvo proporciju atšķirības. Sākot no dienvidu platuma grādiem Latvijā (79,9 % triterpenoīdu no kopējā vaska daudzuma) līdz vietām Somijā (50,7 % triterpenoīdu no kopējā vaska daudzuma) un beidzot ar ziemeļu Norvēģiju (27,4 % triterpenoīdu no kopējā vaska daudzuma), 2018. gadā bija vērojama konsekventa triterpenoīdu īpatsvara samazināšanās tendence ogu vaskā. No dienvidu līdz ziemeļu platuma grādiem bija vērojama tendence vienlaikus palielināties taukskābju un alkānu daudzumam (**7. publikācija**). Atšķirības dažādu platuma grādu ogu kutikulārā vaska sastāvā tika pētītas, izmantojot RDA analīzi, kas parāda saistību starp daudzfaktoriāliem datiem. RDA atklāja negatīvu korelāciju starp triterpenoīdu un taukskābju saturu kutikulārajā vaskā. Triterpenoīdu koncentrācija korelēja ar maksimālo un vidējo temperatūru, bet taukskābju saturs uzrādīja negatīvu korelāciju ar vidējo un maksimālo temperatūru.



7. attēls. RDA, kas parāda saistību starp ogu vaskā konstatētajiem savienojumiem un laikapstākļu raksturlielumiem dažādās ogu ievākšanas vietās 2018. gada vasarā.

Pētījumi par ogu un to spiedpalieku vaskiem papildina zināšanas par augu aizsardzības mehānismiem pret stresu, to sastāva mainīgumu atkarībā no ģeogrāfiskajiem un klimatiskajiem faktoriem.

3.4. Ogu un to spiedpalieku polifenoli

Ogu polifenoli veido lielu sekundāro metabolītu grupu. Polifenoli ir gan ogu miziņā un mīkstumā, gan sēklās. Galvenā polifenolu grupa ogās (jo īpaši intensīvi krāsainās ogās) ir antociāni. Šī disertācijas daļa ir veltīta polifenolu, īpaši antociānu, ekstrakcijai un tās optimizācijai, ekstraktu raksturošanai un iespējamām ekstrakcijas/attīrīšanas metožu un ekstraktu īpašību izmantošanas jomām.

3.4.1. Ogu polifenolu ekstrakcijas optimizācija

Ekstrakcijas šķīdinātāja iespējamā sastāva noteikšana

Pārtikas rūpniecības atkritumu (ogu spiedpalieku) utilizācija ir daudzsološs dabisko antioksidantu – polifenolu – avots. Polifenolu sastāvs augu materiālā ir atkarīgs no augu sugas un to izplatības dažādos audos. Lieli polifenolu daudzumi ir saistīti ogu sēklās un mizā, kas apgrūtina šo savienojumu izdalīšanu. Ekstrakcijas nosacījumus, kas paredzēti vienam augam, nevar tieši izmantot polifenolu ekstrakcijai no cita auga, jo dažādās sugās polifenoli ir specifiski lokalizēti. Tāpēc ir optimizēta ekstrakcijas metode, kas īpaši paredzēta *Vaccinium* ogu spiedpaliekām (**8.** un **9. publikācija**).

Tika apkopota informācija par ekstrakcijas šķīdinātājiem, kas izmantoti polifenolu ekstrakcijai no cita veida materiāliem, un tie tika testēti uz ogu spiedpaliekām, lai noteiktu ekstrakcijas šķīdinātāju, kas nodrošina vislielāko polifenolu un antocianīnu ieguvi (**8.** un **9. publikācija**). Vislielākais ekstrakcijas iznākums (48,38 g/100 g ogu) tika iegūts, izmantojot metanolu un 1 % HCl (v/v). Šī ekstrakcija nodrošināja arī lielāko kopējo antociānu (0,451 g/100 g ogu) un polifenolu (4,8 g/100 g ogu) iznākumu. Tomēr, izmantojot šo sistēmu, jāņem vērā antociānu molekulu stabilitāte. Etanola vienkāršā izmantošana un zemās izmaksas, kā arī augstais polifenolu iznākums, kas iegūts, izmantojot šo šķīdinātāju (3,43 g/100 g ogu), pamato zemāko spirtu (etanola, metanola) izvēli turpmākai ekstrakcijas apstākļu optimizācijai.

Polifenolu ekstrakcijas metodes izvēle un optimizācija

Izvēloties piemērotu ekstrakcijas metodi, var ievērojami palielināt ekstrahēto polifenolu un antociānu daudzumu. Lai noteiktu metodi, kas ir vispiemērotākā ogu spiedpalieku un veselu ogu ekstrakcijai, tika veikta virkne ekstrakciju, izmantojot dažādas ekstrakcijas metodes, vienlaikus izmantojot nemainīgus ekstrakcijas šķīdinātāja parametrus (96 % etanolu, 0,5 % TFA).

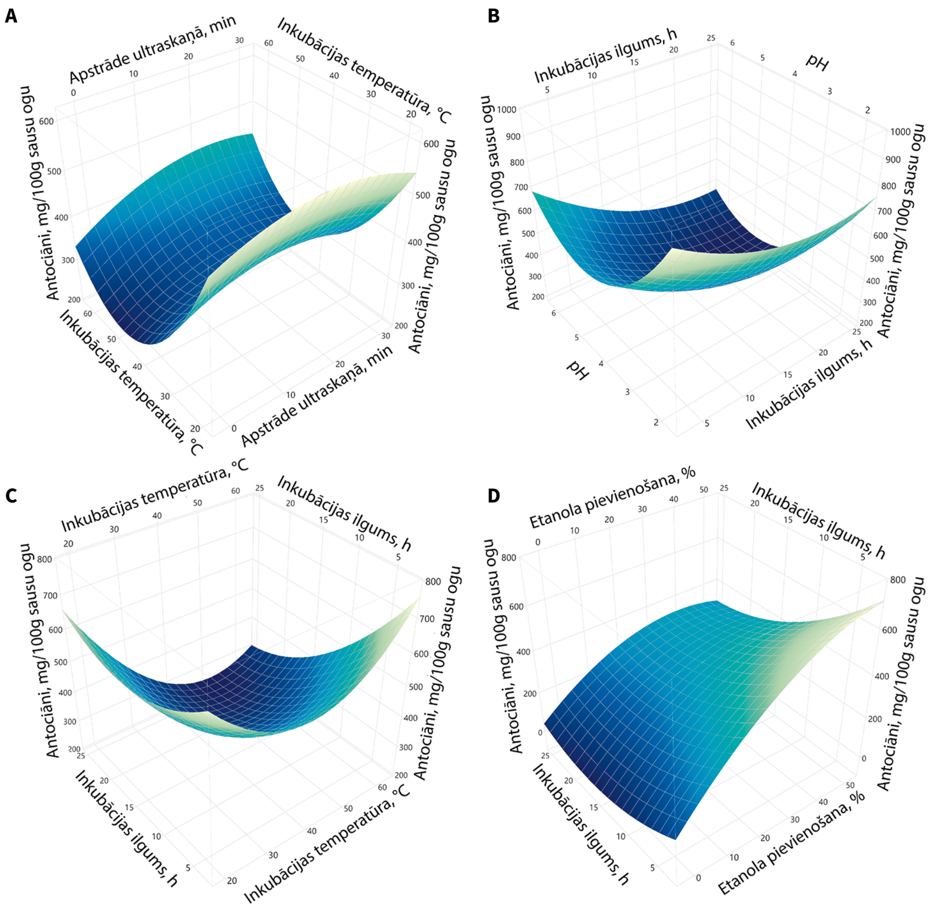
Optimālais ekstrakcijas iznākums tika iegūts, izmantojot ekstrakciju ar ultraskaņas palīdzību, kad visi noteiktie parametri deva augstākos rezultātus. Viszemākais iznākums tika iegūts, izmantojot superkritisko CO_2 ekstrakciju; šī metode ir izmantota polifenolu ekstrakcijai citos pētījumos, tomēr tā ir piemērotāka hidrofobu savienojumu ekstrakcijai.

Šķīdinātāja sastāva optimizācija polifenolu ekstrakcijai, izmantojot RSM pieeju

Neraugoties uz to, ka kopējo polifenolu ekstrakcija ir plaši pētīta, izmantoto ekstrakcijas šķīdinātāju koncentrācija un sastāvs joprojām atšķiras dažādos pētījumos. Nav vienprātības par optimāliem ekstrakcijas parametriem, jo īpaši rūpnieciska vai daļēji rūpnieciska mēroga ekstrakcijai. Statistiskā optimizācija ņem vērā mijiedarbību starp dažādiem mainīgajiem un tās ietekmi uz novēroto reakciju. Izmantojot RSM metodi, tika pētīta etanola vai metanola un skudrskābes vai TFA ietekme uz kopējo polifenolu un antociānu ekstrakcijas efektivitāti. Tika sagaidīts, ka abiem pētītajiem ekstrakcijas efektivitātes rādītājiem (kopējam antociānu un kopējam polifenolu daudzumam) būs cieša korelācija, tomēr RSM metode atklāja, ka antociāniem labvēlīgākie ekstrakcijas apstākļi nav tādi paši kā kopējam polifenolu daudzumam. Maksimāli ekstrahējot antociānus, samazinās kopējais ekstrahēto polifenolu daudzums un otrādi.

Polifenolu ekstrakcijas optimizācija, izmantojot RSM pieeju un enzimatisko hidrolīzi un apstrādi ar ultraskaņu

Lai veicinātu bioloģiski aktīvo savienojumu izdalīšanos no ogu spiedpaliekām, šūnapvalki ir jāsagrauj. Lai noārdītu ogu miziņu šūnapvalku strukturālos polisaharīdus, tādējādi ekstrakcijas vidē atbrīvojot šūnu saturu, var izmantot pektolītiskos enzīmus. Ogu miziņu fizikālās īpašības ļauj šūnu sagraušanai izmantot pektolītiskos enzīmus; tomēr enzīmu hidrolīzes iedarbību varētu uzlabot vēl vairāk, izmantojot ultraskaņu. Enzīmu un ultraskaņas iedarbība tika novērtēta un optimizēta, izmantojot RSM pieeju (8. attēls), lai veicinātu polifenolu savienojumu (īpaši antociānu) izdalīšanos. Iegūtie rezultāti tika izmantoti, lai aprēķinātu optimālos apstākļu parametrus, pamatojoties uz maksimālo polifenolu un antocianīnu ekstrakcijas iznākumu.



8. attēls. Trīsdimensiju atbildes virsmas diagrammas, kurās parādīta ultraskaņas apstrādes ilguma (X_1), etanola pievienošanas (X_2), inkubācijas temperatūras (X_3), pH (X_4) un inkubācijas ilguma (X_5) ietekme uz kopējo antociānu ekstrakcijas iznākumu no melleņu spiedpaliekām. (A) X_1 X_3, (B) X_4 X_5, (C) X_3 X_5 un (D) X_2 X_5 ietekme.

3.4.2. Iegūto polifenolu ekstraktu kvalitatīvā un kvantitatīvā analīze

Vaccinium ogu spiedpalieku ekstrakta sastāvs

Polifenolu ekstrakcijas iznākums pētītajās ogās svārstījās no 2,44 g/100 g spiedpalieku līdz 3,33 g/100 g attiecīgi no lielogu dzērvenēm un meža mellenēm. Rezultāti, kas parādīti 6. tabulā, liecina, ka no katras ogas var iegūt specifiskas polifenolu grupas, piemēram, kopējos procianidīnus (purva dzērvenes) vai antocianīnus (meža mellenes, krūmmellenes) un, iespējams, arī citas polifenolu grupas, par ko liecina kopējais polifenolu saturs sagatavotajos spiedpalieku ekstraktos.

6. tabula. *Vaccinium* ogu spiedpalieku ekstraktu sastāvs.

	Polifenoli*	Kopējie polifenoli**	Procianidīni**	Antociāni**
Lielogu dzērvenes	2,44 ± 0,14	47,9 ± 2,2	0,43 ± 0,02	3,13 ± 0,14
Meža mellenes	3,33 ± 0,18	36, 5 ± 1,6	4,62 ± 0,21	13,3 ± 0,6
Purva dzērvenes	3,05 ± 0,17	48,54 ± 2,18	8,61 ± 0,39	5,95 ± 0,27
Krūmmellenes	3,21 ± 0,18	56,0 ± 2,5	1,91 ± 0,09	34,8 ± 1, 6
Brūklenes	2,63 ± 0,15	52,5 ± 2,4	2,04 ± 0,09	1,59 ± 0,07

* – g/100 g spiedpalieku; ** – g/100 g spiedpalieku ekstrakta. Dati ir vidējie ± SD, *n* = 3.

Vaccinium ogu spiedpalieku ekstraktu polifenolu un antociānu sastāvs

Tika noteikts atsevišķu antociānu saturs pētītajās ogu spiedpaliekās. Kopumā tika konstatēti 15 dažādi antociāni, no kuriem 6 ir raksturīgi sarkanas krāsas ogām (brūklenēm un abām dzērveņu sugām). Antociānu saturs, kā arī polifenolu saturs kopumā ir atkarīgs no ogu spiedpalieku kvalitātes. Lai izvairītos no šo vielu oksidēšanās, spiedpaliekas jāuzglabā saldētas hermētiskos traukos vai maisiņos. Pēc kāda laika antociānu noārdīšanās kļūst acīmredzama – spiedpaliekas sāk brūnēt, kas norāda uz antociānu noārdīšanos. Daži antociāni ir stabilāki par citiem, piemēram, sarkanās krāsas ogās esošie antociāni ir vairāk pakļauti noārdīšanās procesam. Katras ogas antociānu profilus var izmantot arī kā instrumentu autentiskuma pārbaudei, jo katrai ogai ir raksturīgi antociānu profili (7. tabula; **9. publikācija**).

Atsevišķus polifenolus, kas pieder pie dažādām polifenolu grupām, noteica, izmantojot ORBITRAP-HRMS. Atrastās vielas identificēja, salīdzinot atrasto m/z ar aprēķināto potenciālo kandidātu precīzo masu. Dažas vielas apstiprināja, izmantojot autentiskus standartus, ja tādi bija pieejami (**10. publikācija**). Kopumā tika identificēti 216 signāli, kas piederēja pie polifenoliem, papildus vairāk nekā 850 neidentificētiem signāliem, kas tika konstatēti spiedpalieku ekstraktos. Lielais skaits, daudzveidība un autentisku standartu trūkums ierobežo polifenolu savienojumu identifikāciju šādos sarežģītos ekstraktos, neraugoties uz izmantoto augstas izšķirtspējas masspektrometrijas metožu iespējām, precizitāti un izšķirtspēju.

7. tabula. Apkopotie UPLC un LC-TOF kvalitatīvie un kvantitatīvie dati par atrastajiem antociāniem pētītajās *Vaccinium* ogās.

Antociāns	**Daudzums (mg/g spiedpalieku)**[a]				
	Meža mellenes	**Krūmmellenes**	**Purva dzērvenes**	**Lielogu dzērvenes**	**Brūklenes**
Delfinidīna-3-*O*-galaktozīds	31,41 ± 3,06	7,62 ± 0,64	ND	ND	ND
Delfinidīna-3-*O*-glikozīds	39,70 ± 3,22	1,83 ± 0,17	ND	ND	ND
Cianidīna-3-*O*-galaktozīds	26,50 ± 2,28	1,56 ± 0,13	9,81 ± 0,96	1,73 ± 0,17	19,30 ± 1,89
Delfinidīna-3-*O*-arabinozīds	26,27 ± 2,55	6,47 ± 0,53	ND	ND	ND
Cianidīna-3-*O*-glikozīds	49,18 ± 4,21	0,86 ± 0,81 ND	0,71 ± 0,06	0,06 ± 0,01	1,65 ± 0,15
Petunidīna-3-*O*-galaktozīds			ND	ND	
Cianidīna-3-*O*-arabinoside	28,70 ± 2,34	1,15 ± 0,09	9,14 ± 0,89	3,07 ± 0,31	5,44 ± 0,45
Petunidīna-3-*O*-glikozīds	8,95 ± 0,71	6,50 ± 0,60	ND	ND	ND
Peonidīna-3-*O*-galaktozīds	2,73 ± 0,28	0,24 ± 0,02	12,41 ± 1,21	3,04 ± 0,21	0,33 ± 0,04
Petunidīna-3-*O*-arabinozīds	8,55 ± 0,76	5,54 ± 0,44	ND	ND	ND
Peonidīna-3-*O*-glikozīds	20,44 ± 1,91	1,58 ± 0,29 ND	3,28 ± 0,30	0,36 ± 0,04	0,86 ± 0,07
Malvidīna-3-*O*-galaktozīds			ND	ND	
Peonidīna-3-*O*-arabinozīds	9,93 ± 0,86	25,82 ± 2,45	8,18 ± 0,70	2,31 ± 0,23	0,31 ± 0,03
Malvidīna-3-*O*-glikozīds	26,20 ± 2,38	5,34 ± 0,49	ND	ND	ND
Malvidīna-3-*O*-arabinozīds	6,39 ± 0,62	19,61 ± 1,14	ND	ND	ND

[a] Dati izteikti kā vidējās vērtības ± standartnovirze ($n = 3$), ND – nav konstatēts.

3.4.3. Polifenolu ekstraktu pielietojuma identifikācija

Optimizēta veselu ogu un spiedpalieku ekstrakcija

Lai pārliecinātos par optimizēto ekstrakcijas apstākļu izmantošanas pielietojumu citām *Vaccinium* ogām, tās tika ekstrahētas. izmantojot optimizētos ekstrakcijas apstākļus. Lai palielinātu izvēlēto optimālo ekstrakcijas parametru pārbaudes apjomu, izmēģinājumos izmantoja kaltētas ogu spiedpaliekas un veselas kaltētas ogas, kas nav apstrādātas. Šī metode sniedza informāciju par polifenolu saturu abu veidu paraugos, ļaujot noteikt, vai veselām ogām vai ogu spiedpaliekām ir lielāks potenciāls izmantošanai funkcionālu sastāvdaļu un polifenolu koncentrātu iegūšanai. Melleņu, lielogu dzērveņu un purva dzērveņu spiedpaliekās bija ievērojami vairāk polifenolu nekā neapstrādātās veselās ogās (**9. publikācija**).

Veiktie validācijas eksperimenti parādīja, ka optimālie antociānu ekstrakcijas apstākļi deva ievērojami lielāku ekstrakcijas iznākumu salīdzinājumā ar kontroles apstākļiem (vismazāk labvēlīgs šķīdinātāja sastāvs, kā noteikts ar RSM).

Ogu spiedpaliekās ir vairāk antociānu (w/w%) nekā veselās ogās. Šo atšķirību var izskaidrot ar antociānu esamību visā auglī, nevis tikai miziņā. Piemēram, krūmmellenēm ar tumši zilu mizu ir balts vai zaļgans mīkstums un līdz ar to neliels kopējo antociānu daudzums. Turpretī meža mellenēs antociāni ir visā ogā (mizā un mīkstumā) (**9. publikācija**).

Ogu spiedpalieku ekstraktu hipoglikēmiskās un hepatoprotektīvās īpašības

Tika pārbaudītas melleņu, krūmmelleņu, purva un lielogu dzērveņu, brūkleņu spiedpalieku ekstraktu antioksidatīvās, hipoglikēmiskās un hepatoprotektīvās īpašības. Visaugstākā kopējā spēja saistīt brīvos radikāļus bija purva dzērveņu ekstraktiem, kam sekoja melleņu, brūkleņu, lielogu dzērveņu un krūmmelleņu ekstrakti. Dzērveņu un lielogu dzērveņu ekstraktiem bija tādas pašas DPPH saistīšanas spējas kā askorbīnskābei, dzērveņu ekstraktiem bija vislielākā spēja saistīt brīvos radikāļus. Meža melleņu ekstraktam bija vislielākā superoksīda dismutāzes aktivitāte, dzērveņu ekstraktam tā bija divas reizes mazāka. Visi ekstrakti uzrādīja no koncentrācijas atkarīgu aizsargājošu iedarbību pret tercbutilhidroperoksīda izraisītu citotoksicitāti un ievērojamus HepG2 šūnu izdzīvošanas rādītājus 0,25 mg/mL koncentrācijā, visaugstākā aktivitāte bija dzērveņu ekstraktam. Visiem pieciem ogu spiedpalieku ekstraktiem bija inhibējoša ietekme uz amilāzes un glikozidāzes aktivitāti, turklāt glikozidāzes aktivitāte tika inhibēta efektīvāk. Iegūtie rezultāti liecina, ka *Vaccinium* ogu izspaidu ekstraktiem piemīt spēcīga antioksidanta spēja un tie inhibē ogļhidrātu transformācijā gremošanas sistēmā iesaistītos enzīmus, tos var potenciāli izmantot ar oksidatīvo stresu saistītu hronisku slimību profilaksē (**10. publikācija**).

Ogu spiedpalieku ekstraktu izraisīta NF-κB ceļa un COX-2 aktivitātes inhibīcija

Tika pētītas ogu spiedpalieku ekstraktu pretiekaisuma īpašības, izmantojot uz šūnām balstītu testu. Ekstraktiem piemita spēja inhibēt enzīmu ciklooksigenāzi-2 (COX-2) un samazināt proiekaisuma citokinīnu izdalīšanos monocītiskajās THP-1 šūnās, kas stimulētas ar baktēriju lipopolisaharīdu (LPS). Rezultāti parādīja, ka visiem ekstraktiem bija no koncentrācijas atkarīga COX-2 inhibējoša iedarbība ar vairāk nekā 50 % inhibīciju, ja to koncentrācija bija 1 mg/mL. Viszemākā inhibējošā iedarbība tika atrasta meža melleņu ekstraktam, savukārt krūmmelleņu ekstrakts nodrošināja visaugstāko inhibīciju zemākā testētā koncentrācijā. Ekstrakti būtiski neietekmēja THP-1 šūnu dzīvotspēju, ja to koncentrācija bija 1 mg/mL vai mazāka. Ekstrakti arī samazināja transkripcijas faktora NF-kB kodola translokāciju, kas ir iesaistīts iekaisuma veidošanās procesā, un mazināja proiekaisuma citokinīnu ekspresiju LPS stimulētās THP-1 šūnās. Šie secinājumi liecina, ka *Vaccinium* ģints ogu spiedpalieku ekstraktiem piemīt pretiekaisuma iedarbība un tie var būt noderīgs bioaktīvo komponentu avots veselīgam uzturam (**11. publikācija**).

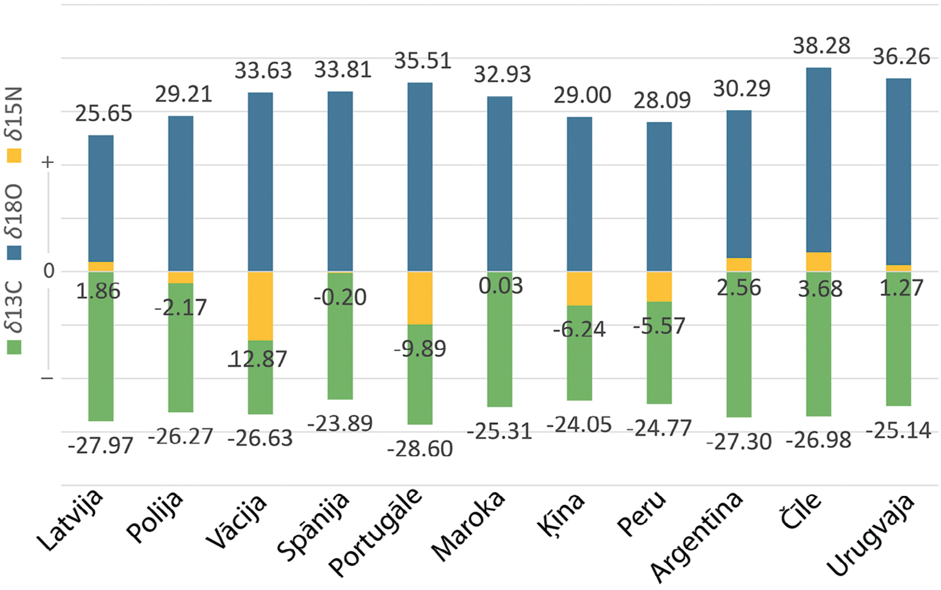
Enzīmus apstrādājot ar ultraskaņu, var iegūt melleņu un brūkleņu sulu ar labākām īpašībām

Pētījumā tika pārbaudīta fermentu un ultraskaņas izmantošana, lai iegūtu krūmmelleņu un brūkleņu sulu ar paaugstinātu antioksidantu aktivitāti un izteiktāku krāsu. Rezultāti parādīja, ka, apstrādājot ar ultraskaņu, palielinājās abu ogu sulas iznākums, bet ietekme bija nozīmīgāka, ja tika izmantots arī enzīms. Kombinētajā apstrādē palielinājās sulas sausnas saturs, kas norāda uz šķīstošo savienojumu izdalīšanos. Arī kopējais polifenolu saturs un antioksidatīvā aktivitāte palielinājās, apstrādājot ar enzīmu, lielāks pieaugums bija vērojams brūkleņu sulā. Kombinētajā apstrādē uzlabojās arī sulas vizuālais izskats, tā bija dzidra un aromātiska. Enzimātiskā apstrāde palīdzēja arī atrisināt problēmas ar brūkleņu sulas viskozitāti un filtrējamību. Ar enzīmiem apstrādātai melleņu biomasai pievienojot skābāku sulu, piemēram, brūkleņu sulu, varētu palielināt sulas un kopējo polifenolu savienojumu daudzumu, jo enzīmu aktivitāte ir augstāka zema pH gadījumā(**12. publikācija**).

3.5. Stabilo izotopu un mikroelementu saturs kā autentiskuma un izsekojamības rīks

Vaccinium ģints ogu, tostarp kultivēto krūmmelleņu (*Vaccinium corymbosum* L.) un savvaļas melleņu (*Vaccinium myrtillus* L.), patēriņš laika gaitā ir pastāvīgi palielinājies. Tāpēc šo ogu sastāva pētījumi ir īpaši svarīgi, ņemot vērā to plašo izmantošanu etnomedicīnā, sulu un ievārījumu ražošanā, kā funkcionālo pārtikas produktu, tostarp ekstraktu. pagatavošanā, kurus varētu izmantot farmācijas un kosmētikas nozarē. Pieaugot patēriņam, var rasties produktu viltojumi un nezināmas izcelsmes produkti. Šā pētījuma mērķis bija raksturot elementu (izmantojot ICPOES) un vieglo stabilo izotopu sastāvu (IRMS), kā arī elementu koncentrācijas atšķirības meža mellenēs, kas ievāktas dažādās Ziemeļeiropas vietās, un komerciāli pieejamos krūmmelleņu paraugos no visas pasaules (9. attēls). Iegūtie rezultāti liecina, ka pēc makroelementu un mikroelementu satura mellenēs var noteikt to izcelsmes vietu.

Rezultāti, kas iegūti par dažādu krūmmelleņu šķirņu elementu sastāvu, kuras ievāktas vienā un tajā pašā laukā, liecina, ka elementu sastāvs lielā mērā ir atkarīgs no melleņu šķirnes, nevis no augsnes elementsastāva. Tika konstatēts, ka mikroelementu līmenis komerciāli pieejamos ogu paraugos ir zems. Lielāka elementu koncentrācijas līmeņu izkliedētība mellenēs norāda uz iespēju izmantot elementsastāva analīzes datus autentiskuma pētījumos, tomēr vispirms būtu jānosaka atsauces vērtības, lai analīžu rezultātus piesaistītu konkrētām ģeogrāfiskām vietām vai ģeomorfoloģiskajiem apstākļiem (**13. publikācija).**



9. attēls. Vieglo stabilo elementu (δ^{13}C, δ^{15}N, δ^{18}O) izotopu attiecības vērtības krūmmellenēs no dažādām valstīm.

Elementu sastāvu un stabilo izotopu attiecības var izmantot kā instrumentu meža melleņu izsekojamībai

Meža mellenes tika ievāktas visā Latvijā, kā arī Norvēģijā, Lietuvā un Somijā. Latvijā savāktās mellenes tika salīdzinātas, lai novērotu vietējās (64 000 km^2 robežās) elementu sastāva izmaiņas. Konstatētās paaugstinātās elementu koncentrācijas norādīja uz vietējām vai reģionālām vides piesārņojuma vietām. Ņemot vērā Latvijā konstatētos ģeoķīmiskos apstākļus un vispārējo augsnes sastāva viendabīgumu, šie rezultāti liecina, ka elementu koncentrāciju varētu izmantot kā autentiskuma noteikšanas līdzekli, lai atšķirtu ogas, kas augušas dažādos reģionos.

Dati, kas iegūti, veicot metālu un vieglo, stabilo izotopu analīzi Somijā, Latvijā, Lietuvā un Norvēģijā ievāktajās mellenēs, tika analizēti, izmantojot PCA, lai vizualizētu iespējamās atšķirības starp ogu novākšanas reģioniem. PCA parādīja, ka katrā atsevišķā valstī veidojas klasteris, pamatojoties uz elementu un stabilo izotopu attiecību kopumu. Tika analizēti 24 elementi, kas kombinācijā ar vieglo, stabilo izotopu attiecībām ļāva atšķirt Baltijas jūras reģiona valstīs un Norvēģijā augušās meža mellenes, līdz ar to elementsastāva un izotopu sastāva analīzi var izmantot kā ogu izcelsmes autentiskuma pārbaudes līdzekli (**13. publikācija**).

Secinājumi

1. Priekšnosacījums bioekonomikas mērķu sasniegšanai ir biomasas (atbilstoši aprites bioekonomikas koncepcijai vēlams, izmantojot biomasas atkritumus) pārstrādes pieeju (stratēģijas) izstrāde, ņemot vērā iegūto vielu un materiālu sastāvu, pielietošanas iespējas un tirgus vajadzības. Tādējādi biomasas sastāva padziļināta analīze nosaka biorafinēšanas pieejas attīstības virzienu un vietu biorafinēšanas vērtību piramīdā, kā arī atbalsta pārstrādes metožu izstrādi kā galveno faktoru, kas ietekmē biomasas biorafinēšanas panākumus.
2. Ogu lipīdi ir potenciāli viena no vērtīgākajām ogās esošajām, veselību veicinošo savienojumu grupām, un tiem var būt nozīmīga loma jaunu, lietotājam draudzīgu, inovatīvu produktu izstrādē. *Vaccinium* ogu, to spiedpalieku un ogu lipīdu ekstrakcijas iespēju pētījumi atklāj, ka ekstrakcijas iznākums un ekstrakta sastāvs ir ļoti atkarīgs no izmantotā ekstrakcijas šķīdinātāja, un ir vēlams izmantot intensīvas ekstrakcijas metodes, galvenokārt videi draudzīgus ekstrakcijas šķīdinātājus. Izstrādātā ekstrakta frakcionēšanas metode liecina par potenciālu iegūt lipīdu grupas ar funkcionāli izmantojamu sastāvu, tādējādi paplašinot lipīdu ekstrakta izmantošanas iespējas. Plašais ogu lipīdos konstatēto savienojumu grupu spektrs padara tos pievilcīgus kā antibakteriālus līdzekļus dažādiem pielietojumiem pārtikas produktu konservēšanā, profilaktiskos palīglīdzekļos, kā daļu no uztura bagātinātājiem, kosmētikā, un, iespējams, tie varētu samazināt tradicionālo antibiotiku lietošanu. Iegūtās saules aizsardzības faktora vērtības liecina, ka ogu lipīdu izmantošanai ir potenciāls saules aizsarglīdzekļu ražošanā, tādējādi aizstājot sintētiskās un neorganiskās saules aizsarglīdzekļu sastāvdaļas ar dabiskām un ilgtspējīgām sastāvdaļām.
3. Ogu epikutuláro, virsmas vasku un to spiedpalieku izpēte ļāva identificēt galvenos ogu vasku komponentus un papildināja iegūtās zināšanas par vasku kā nozīmīgu augu aizsargmehānismu elementu pret stresu. Vaska sastāva analīze, izmantojot dažādas metodes, atklāja epikutikulārā vaska morfoloģiju, komponentu mainību atkarībā no ģeogrāfiskajiem un klimatiskajiem faktoriem, norādot uz potenciālo augu reakciju uz klimata pārmaiņu ietekmi. Padziļināta ogu vaska sastāva izpēte un videi draudzīgas ekstrakcijas iespēju demonstrēšana, atbalstot no ogu sulas ražošanas atkritumiem iegūtā vaska izmantošanas iespējas, iezīmē vasku pielietojuma potenciālu.
4. Padziļināta *Vaccinium* ogu un to spiedpalieku polifenolu izpēte parādīja to daudzpusību un unikalitāti salīdzinājumā ar citiem augiem, kā arī nozīmi funkcionālo sastāvdaļu vai produktu izstrādē. Polifenolu ekstrakcijas optimizācija, izmantojot atbildes virsmas metodoloģiju (*Response Surface Methodology*), kā arī enzimātiskās hidrolīzes izmantošana ekstrakcijai pavēra iespējas iegūt specifiskas polifenolu grupas ar augstu iznākumu, izmantojot videi draudzīgas ekstrakcijas sistēmas. Turpmākā frakcionēšanas gaitā tika pirmo reizi identificēti polifenoli, kas iepriekš nebija konstatēti *Vaccinium* ogu sastāvā un kam piemīt augsta bioloģiskā (farmakoloģiskā) aktivitāte, par ko liecina to brīvo radikāļu saistīšanas spēja, pretiekaisuma, hepatoprotektīvā, hipoglikēmiskā un citas aktivitātes.
5. Ogu elementu sastāva analīze, vieglo, stabilo elementu izotopu attiecības noteikšana, kā arī ogu un to spiedpalieku lipīdu un polifenolu sastāva analīze palīdz identificēt to izcelsmi, tādējādi nodrošinot autentiskuma un izsekojamības uzdevumus.

6. Pilnīgu bezatkritumu stratēģiju varētu izstrādāt, izmantojot ierosinātās biorafinēšanas pieejas papildus citām biomasas konversijas procedūrām, kas ir veiksmīgi demonstrētas un izmantotas cita veida lielapjoma pārtikas rūpniecības atkritumiem. No pārtikas atkritumiem varētu iegūt īpašas savienojumu grupas, un izlietoto biomasu varētu pārveidot enerģijā vai biomasā esošo oglekli pārvērst biooglē un izmantot lauksaimniecībā, lai panāktu klimata neitralitāti, tādējādi īstenojot aprites ekonomikas principus.

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Pielikumi – rakstu krātuve

ARTICLE 1

Gas chromatography–mass spectrometry study of lipids in northern berries

L. Klavins, J. Kviesis, I. Steinberga, L. Klavina and M. Klavins
Agronomy Research, 14, 1328–1346

ARTICLE 2

Lipids of cultivated and wild *Vaccinium* spp. berries from Latvia

L. Klavins, A. Viksna, J. Kviesis, M. Klavins
Proceedings of the 13th Baltic Conference on Food Science and Technology, 198–203

DOI: 10.22616/FoodBalt.2019.019

ARTICLE 3

Composition, sun protective and antimicrobial activity of lipophilic bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.) extract fractions

L. Klavins, M. Mezulis, V. Nikolajeva, M. Klavins
LWT – Food Science and Technology, 138, 110784

https://doi.org/10.1016/j.lwt.2020.110784

ARTICLE 4

Compositional and morphological analyses of wax in northern wild berry species

P. Trivedi, K. Karppinen, L. Klavins, J. Kviesis, P. Sundqvist, N. Nguyen, E. Heinonen, M. Klavins, L. Jaakola, J. Väänänen, J. Remes, H. Häggman

Food Chemistry, 295, 441–448

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ARTICLE 5

Cuticular wax composition of wild and cultivated northern berries

L. Klavins, M. Klavins

Foods, 9 (5), 587

https://doi.org/10.3390/foods9050587

ARTICLE 6

Analysis of composition, morphology, and biosynthesis of cuticular wax in wild type bilberry (*Vaccinium myrtillus* L.) and its glossy mutant.

P. Trivedi, N. Nguyen, L. Klavins, J. Kviesis, E. Heinonen, J. Remes, S. Jokipii-Lukkari, M. Klavins, K. Karppinen, L. Jaakola, H. Häggman

Food Chemistry, p.129517

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ARTICLE 7

Temperature has a major effect on the cuticular wax composition of bilberry (*Vaccinium myrtillus* L.) fruit

P. Trivedi, L. Klavins, A. L. Hykkerud, J. Kviesis, D. Elferts, I. Martinussen, M. Klavins, K. Karppinen, H.M. Häggman, L. Jaakola

Frontiers in Plant Science, p.3497

https://doi.org/10.3389/fpls.2022.980427

ARTICLE 8

Comparison of methods of extraction of phenolic compounds from American cranberry (*Vaccinium macrocarpon* L.) press residues

L. Klavins, J. Kviesis, M. Klavins

Agronomy Research, 15(2), 1316–1330

ARTICLE 9

Berry press residues as a valuable source of polyphenolics: Extraction optimization and analysis

L. Klavins, J. Kviesis, I. Nakurte, M. Klavins

LWT – Food Science and Technology, 93, 583–591

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ARTICLE 10

Antioxidative, hypoglycaemic and hepatoprotective properties of five *Vaccinium* spp. berry pomace extract

R. Muceniece, L. Klavins, J. Kviesis, K. Jekabsons, R. Rembergs, K. Saleniece,
Z. Dzirkale, L. Saulite, U. Riekstina, M. Klavins

Journal of Berry Research, 9(2), 267–282

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ARTICLE 11

Inhibition of NF-κB pathway in LPS-stimulated THP-1 monocytes and COX-2 activity in vitro by berry pomace extracts from five *Vaccinium* species

L. Kunrade, R. Rembergs, K. Jekabsons, L. Klavins, M. Klavins,
R. Muceniece, U. Riekstina

Journal of Berry Research, 10(3), 381–396

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ARTICLE 12

Optimisation of blueberry (*Vaccinium corymbosum* L.) press residue extraction using a combination of pectolytic enzyme and ultrasound treatments.

L. Klavins, E.P. Puzule, J. Kviesis, M. Klavins

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ARTICLE 13

Trace Element Concentration and Stable Isotope Ratio Analysis in Blueberries and Bilberries: A Tool for Quality and Authenticity Control

L. Klavins, I. Maaga, M. Bertins, A.L. Hykkerud, K. Karppinen, Č. Bobinas,
H. M. Salo, N. Nguyen, H. Salminen, K. Stankevica, M. Klavins

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