Eesti Maaülikool
Estonian University of Life Sciences

VALORISATION OF FRUITS OF ROWAN (*SORBUS* SPP.) GENOTYPES FOR FUNCTIONAL FOOD INGREDIENTS

PIHLAKA (*SORBUS* SPP.) GENOTÜÜPIDE VILJADE VÄÄRINDAMINE FUNKTSIONAALSE TOIDU KOOSTISOSADEKS

VIIVE SARV

A Thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2023

Eesti Maaülikooli doktoritööd

Doctoral Theses of the Estonian University of Life Sciences

"Science is not only a disciple of reason but also one of romance and passion."
Stephen Hawking

Eesti Maaülikool
Estonian University of Life Sciences

VALORISATION OF FRUITS OF ROWAN (*SORBUS* SPP.) GENOTYPES FOR FUNCTIONAL FOOD INGREDIENTS

PIHLAKA (*SORBUS* SPP.) GENOTÜÜPIDE VILJADE VÄÄRINDAMINE FUNKTSIONAALSE TOIDU KOOSTISOSADEKS

VIIVE SARV

A Thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2023

Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences

According to verdict No 6-14/10-1 (19.10.2023) the Doctoral Committee of the Agricultural Sciences of the Estonian University of Life Sciences has accepted the thesis for the defense for the degree of Doctor of Philosophy in Agriculture.

Opponent: **Prof. Marina Heinonen**
University of Helsinki

Supervisors: **Prof. Petras Rimantas Venskutonis**
Estonian University of Life Sciences
Kaunas University of Technology

Prof. Rajeev Bhat
Estonian University of Life Sciences

Dr. Reelika Rätsep
Estonian University of Life Sciences

Defence of the thesis: Estonian University of Life Sciences, Fr. R. Kreutzwaldi 5, Tartu, 14th December 2023 at 09:15 am.

The English language was edited by the Luisa Translation Agency and the Estonian language was edited by the Language Centre of Estonian University of Life Sciences.

Publication of this thesis is supported by the Estonian University of Life Sciences.

ISSN 2382-7076
ISBN 978-9916-719-36-7 (print)
ISBN 978-9916-719-37-4 (pdf)

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals (**I-III** in the text).

I **Sarv, V.**, Venskutonis, P.R., Bhat, B. 2020. The *Sorbus* spp.—Underutilised Plants for Foods and Nutraceuticals: Review on Polyphenolic Phytochemicals and Antioxidant Potential. *Antioxidants* 9, 813: 1-23. doi:10.3390/antiox9090813

II **Sarv, V.**, Venskutonis, P.R., Rätsep, R., Aluvee, A., Kazernavičiūtė, R., Bhat, R. 2021. Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (*Sorbus aucuparia L.* L.) Cultivated in Estonia. *Antioxidants* 10, 1779: 1-15. doi.org/10.3390/antiox10111779

III **Sarv, V.**, Kerner, K., Venskutonis, P.R., Rocchetti, G., Pier Paolo Becchi, P.P., Lucini, L., Tänavots, A., Bhat, R. 2023. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs Food Chemistry: X, 19, 100761. doi.org/10.1016/j.fochx.2023.100761

The contributions of the authors to the papers:

Paper	**I**	**II**	**III**
Conceptualization	VS	VS	VS
Formal analysis	VS	VS; AA; RK	VS; KK; GR; PPB
Investigation	VS	VS	VS; KK; GR
Methodology	VS; PRV	VS; PRV; RB; RR; RK; AA	VS; KK; PRV; GR; PPB; LL
Visualization	VS	VS	AT; GR
Writing – original draft	VS; PRV	VS; PRV	VS; GR
Supervision	RBV; RB	PRV; RB; RR	PRV; RB; LL
Resources	RB	RB	RB

VS-Viive Sarv, PRV- Petras Rimantas Venskutonis, RB-Rajeev Bhat, RR-Reelika Rätsep, AA-Alar Aluvee, RK-Rita Kazernavičiūtė, KK-Kristi Kerner, GB- Gabriele Rocchetti, PPB- Pier Paolo Becchi, LL- Luigi Lucini, AT- Alo Tänavots

ABBREVIATIONS

ABTS•+ — 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate radical
AC — rowanberry pomace after supercritical fluid CO_2 extraction
ACY — anthocyanin
ANOVA — analysis of the variance
AUC — area under the fluorescence decay curve
avr — average
aw— water activity
3-CQA — 3-O-caffeoylquinic acid, chlorogenic acid
5-CQA — 5-O-caffeoylquinic acid, neochlorogenic acid
C — control
Cv(s) — cultivar(s)
Cy_ gal — cyanidin-3-galactoside
Cy_ glu — cyanidin-3-O-glucoside
Cy_ ara — cyanidin-3-arabinoside
DCM — dichloromethane
DPPH• — 2,2-diphenyl-1-picrylhydrazyl radical
dw(e) — dry weight of extract
E — extract
EFSA — *European Food Safety Authority*
ET — electron transfer
FC — Folin–Ciocalteu
Fl — flavonoids
FLAVO — flavonol
FRAP — ferric reducing antioxidant power
FW — fresh weight
GAE — gallic acid equivalents
GRAS — generally regarded as safe
HAT -— hydrogen atom transfer
HCA — hydroxycinnamic acid
HESI — heated electrospray ionization
HF — heavier fraction

HRMS — high-resolution mass spectrometry

HSD — honestly significant difference

LF — lighter fraction

L – linoleic acid

Ln – linolenic acid

LPO — inhibition of lipid peroxidation

MAE — microwave-assisted extraction

O — oleic acid

ORAC — oxygen radical absorbance capacity

OPLS-DA — orthogonal projections to latent structures discriminant analysis

P — palmitic acid

P_C1 — procyanidin C1

PCA — Principal Component Analysis

PUFA — polyunsaturated fatty acid

Qgal — quercetin-3-O-galactoside

Qglu — quercetin 3-glucoside

Qrut — quercetin-3-O-rutinoside

r — linear correlation coefficient

R — residue

S — stearic acid

SFE-CO_2 — supercritical CO_2 extraction

TAG — triacylglycerol

TEAA — Trolox equivalent antioxidant activity

TPC — total phenolic content

UPLC-Q-TOF-MS — ultra-high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry

VIP — variables importance in projection

1. INTRODUCTION

In recent years, researchers have been looking for novel plant-based sources of bioactive compounds. These components are of interest to both, health and nutrition professionals. For instance, the global market of polyphenols is prognosticated to be USD 2.9 billion by 2030 *(Polyphenols Market Growth & Trends, 2022)*. Health benefits of various berries and their products have been reported in numerous articles mainly due to their high content of antioxidant polyphenols, vitamins, minerals, dietary fibres, natural sugars and unsaturated fatty acids. The positive correlation between the high concentration of polyphenols and antioxidant activity have been reported by several authors (Jacobo-Velázquez & Cisneros-Zevallos, 2009; Sadeer *et al.*, 2020). However, from an enormous number of different plant species in the 'Plant Kingdom', there remains a vast number of scarcely investigated plants, which could be used as potential sources of nutraceuticals and functional foods.

The fruits of *Sorbus* species called rowanberries have rather limited applications, as they are not consumed as fresh fruits, mainly due to their astringent flavour. The rowan, also known as whitebeam or mountain ash belongs to the large genus of *Sorbus* L. (*Maloideae, Rosaceae*). The deciduous shrubs or trees of rowans are native throughout the cool temperate regions of the Northern Hemisphere and which tolerate the poor growing conditions. The fruit, the lustrous bark, mostly toothed leaflets as well as convex panicles of rowan species, contain valuable phytochemicals and have been used in traditional medicine (Robertson *et al.*, 1991a). Recently, the aqueous extract of the most well-known and widespead of species of rowan in Europe and Estonia, namely *S. aucuparia L.* or wild rowanberry or European rowan has exhibited a remarkable antibacterial activity against 5 tested bacteria: *Bacillus subtilis subsp. Spizizenii* ATCC 6633, *Bacillus licheniformis* ATCC 14580, *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 9027, and *Klebsiella pneumoniae* ATCC 33495 (Erbil, 2022). Moreover, the fruits of rowan species are an excellent source of polyphenols with a high antioxidant capacity (Sołtys *et al.*, 2020). In addition, it is expected that the other important role of plant-based ingredients possessing antioxidant capacity could be the protection of foods and other sensitive to oxidation products during processing and storage in order to extend their storage time and improve the quality and safety (Bernini *et al.*, 2017).

Recently, the *S. aucuparia* L. extracts were applied as additives in fresh orange juice, where the extracts demonstrated the antimicrobial activity against gram-positive and gram-negative bacteria (Chaimaa, 2021). Due to the significant content of bioactive phenolics, such as *phenolic* acids, anthocyanins and flavonoids in fruits, the preparations of *S. aucuparia* L., have inhibited lipid oxidation both in liposomes and in emulsions (Hukkanen *et al.*, 2006). However, in order to increase the lipophilicity of plant-based polyphenolic substances, some studies have applied for derivatisation of phenolic compounds by attaching long chain alkyl molecules(Aladedunye *et al.*, 2015; Zhong & Shahidi, 2011). For instance, the lipophilised *S. aucuparia L.* phenolic extract, which consisted of approx. 87% chlorogenic acid, has more effectively inhibited rapeseed oil oxidation during 7-day storage compared to the untreated one, by reducing the peroxide value by 43% and improving the solubility of the phenolics during frying (Aladedunye *et al.*, 2015).

Based on the previous studies, the current doctoral thesis focused on applying biorefining concept to underexplored fruits of genotypes cultivated in Estonia for their valorisation in order to develop valuable functional ingredients for foods, nutraceuticals or cosmetics. In order to investigate the background of the genus *Sorbus* L., a review article (**I**) was compiled, which revealed the important aspects of the underexplored rowan genotypes, based on which the thesis plan was prepared.

The rowanberries have traditionally been used for producing juice and alcoholic beverages. Juicing different fruits produces a significant amount of press residue, called pomace, which similarly to the other organic waste, can emit the greenhouse gases, especially methane (CH_4), if it is discarded to the landfills. However, the fruit pomace could be a potential source of several phytochemicals for food or non-food applications, as it has been found that there is a substantial amount of polyphenols (phenolic acids, flavonoids and anthocyanins) in pomace constituents, the seeds, skins and pulp, 60-70%, 28-35% and 10%, respectively (De Ancos *et al.*, 2015). Therefore, extensive research on the assessment of the nutritional value of fruit pomace has been the challenging task of researchers in recent decades (Venskutonis, 2020b). In order to develop functional ingredients out of the rowanberry pomace, the detailed information of the composition and antioxidant properties of this promising natural source would be essential. Therefore, the polyphenolic composition and antioxidant capacity were evaluated for pomace as well

as (for comparison) for fruit and juice samples of 16 sweet rowanberry cultivars (cvs) and wild rowanberry (*S. aucuparia* L. or European rowan) **(II).** The *in vitro* free radical scavenging potential of these plant materials were evaluated by using free radical scavenging capacity methods. Subsequently, the most promising three cultivars with the highest antioxidant capacity and total polyphenolic contents (TPC) were chosen for further biorefining and application as ingredients possessing antioxidant capacity for inhibiting the lipid oxidation in meatballs and prolonging their storage time. Therefore, supercritical fluid extraction (SFE) using CO_2 as a solvent and microwave-assisted extraction (MAE) were applied for valorization of lyophilised and ground rowanberry pomace material, in order to obtain rowanberry pomace-based functional ingredients for meatballs **(III)**. In addition to conventional quality characterization, the untargeted metabolomics was also applied in order to study the effect of rowanberry pomace ingredients in meatballs. When the hydrophilic fraction of pomace contains most of the polyphenolic antioxidants, such as phenolic acids, anthocyanins and flavonoids, the lipophilic seed extracts could be beneficial as nutraceutical and cosmetic agents due to their high content of polyunsaturated fatty acids (omega-3 and omega-6) and carotenoids (Bobinaite *et al.*, 2020). The present research applies a multi-disciplinary approach with concepts of agri-food technology, biotechnology and nutrition sciences being combined with a sustainability approach. This study provides the opportunities for development of rowanberry valorization schemes. Selected physical and chemical methods were tested, focusing on a combination of conventional, high pressure (supercritical fluid extraction), microwave-assisted extraction as well as fractionation methods. Besides being beneficial for local agri-food industries in Estonia and beyond, this work can also benefit the local consumers to understand the benefits of rowan fruits and their applicability for effective processing. In addition, an important aspect is to spread the understanding of the necessity of biorefining of agricultural by-products in order to save the resources and reduce the environmental impact.

2. LITERATURE REVIEW

2.1. Effect of origin and cultivar on biochemical composition of rowan fruits

2.1.1. Botanical classification of genus *Sorbus* L. and the traditional utilisation of the species

The genus *Sorbus* L. includes nearly 250 species, of which 35 are located in the Caucasus and Turkey, 90 in Europe and 110 in Asia (Robertson *et al.*, 1991a). The *Sorbus* L. genus is in turn divided into 6 subgenera (subg.): *Sorbus, Aria, Micromeles, Cormus, Tominaria*, and *Chamaemespilus* (Fig. 1.). Considering their appearance and growth area, these subgenera differ remarkably. For instance, while subg. *Aria, Micromeles, Torminaria* and *Chamaemespilus* have simple leaves and pomes with groups of tanniferous cells; *Sorbus* and *Cormus* have compound leaves, but only *Cormus* has pomes without starch and only *Sorbus* fruit have all the non-tanniferous flesh cells (Aldasoro *et al.*, 1998).

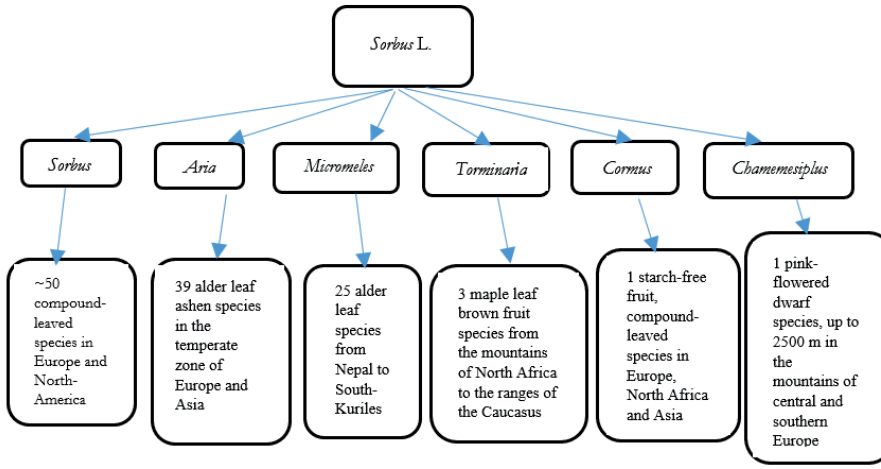


Figure 1. The subgenera of *Sorbus* L., the number, description and the habitat of the species

The astringent fruits of wild rowanberries can acquire a red, orange, yellow, pink or white color in fully ripe sage, and fruit flesh is quite homogenous (Robertson *et al.*, 1991b). The maximum yield of a rowan tree is up to 20 kg (Poyrazoğlu, 2004).

In folk medicine, people have used rowanberries in small amounts as appetizers and for stimulating production of gastric acid, as well as being used as anti-inflammatory, anti-diarrheal, diuretic, vasodilatory agents and a source of ascorbic acid (vitamin C)(Fomenko *et al*., 2016). Moreover, in traditional medicine the rowanberries have been used against rheumatism, constipation and kidney diseases, and the juice against throat hoarseness (Miletic & Paunovic, 2012). In some countries rowanberries have also been used as remedies against intestinal obstructions and against liver and gallbladder diseases (Fomenko *et al*., 2016) (Table 1).

Table 1. Potential uses of different plant parts of rowan species

Berries	Food, medicine, cosmetics, fodder, natural dyes	*S. aria* (L.) Crantz, *S. aucuparia* L., *S. cashmiriana* Hedl., *S. commixta Hedl., Cormus domestica* (L.) Spach, *S. intermedia, S. pohuashanesis, S. sambucifolia* (Cham. et Schltdl.) M.Roem., *S. scopulina* Greene, *S. torminalis* (L.) Crantz, *S. norvegica* Hedl.
Leaves	medicine, cosmetics, fodder	*S. aria* (L.) Crantz, *S. aucuparia* L., *S. commixta Hedl., S. gracilis* (Siebold et Zucc.) K.Koch, *S. koehneana* C.K.Schneid., *S. sambucifolia* (Cham. et Schltdl.) M.Roem., *S. setschwanensis* (C.K.Schneid.) Koehne, *S. tianschanica* Rupr., *S. pogonopetala, S. wilfordii* Koehne
Inflorescences	medicine, cosmetics	*S. aria* (L.) Crantz, *S. aucuparia* L., *S. commixta Hedl., S. gracilis*(Siebold et Zucc.) K.Koch, *S. intermedia, S. decora* (Sarg.) C.K.Schneid., *S. koehneana* C.K.Schneid., *S. sambucifolia* (Cham. et Schltdl.) M.Roem., *S. scalaris* Koehne, *S. sitchensis, S. pohuashanesis*
Bark, twigs	medicine, cosmetics, natural dyes	*S. alnifolia* (Siebold et Zucc.) K.Koch, *S. americana* Marshall, *S. commixta Hedl., S. cashmiriana* Hedl., *S. decora* (Sarg.) C.K.Schneid., *Cormus domestica* (L.) Spach, *S. pohuashanesis, S. tianschanica* Rupr.

*The references for every particular species are in Table 1 in paper **I**

The (subg.) *Cormus* with one species, *Cormus domestica* (L.) Spach, grows in Europe, North Africa and Asia. It has been reported that the fruits of *Cormus domestica* (L.) Spach are traditional anti-inflammatory, antidiarrheal

(dried), antidiabetic, diuretic, vasodilatory agents and vitamin source (Majić *et al.*, 2015). The subg. *Chamaemespilus* (false medlar or dwarf whitebeam), which grows at an altitude of 2500 m in the mountains of central and southern Europe, stands out among other rowan species by a rather distinct flower shape and pink colour (Robertson *et al.*, 1991a). The subg. *Micromeles* includes 25 trees or bushes spread from Nepal to the South Kuriles. In the Korean folk medicine, the twigs of the most widely distributed species, *S. alnifolia* (Siebold & Zucc.) K. Koch., were used for treating neurological disorders (Cheon et al., 2017). The subg. *Torminaria* has three maple-leaved species with brown fruit, growing in the temperate zone of Europe, from the mountains of North Africa to the ranges of the Caucasus. The fruits of *S. torminalis* (L.) Crantz have been traditionally used as diuretic or anti-inflammatory, antidiarrheal (in dried form), vasodilatory remedy and as a source of vitamins, especially vitamin C and E (Aldasoro *et al.*, 2004; Mrkonjić *et al.*, 2017). The subg. *Aria* includes 39 species native to most of Europe as well as North Africa and temperate Asia. Traditionally, the berries of *S. aria* (L.) Crantz have been used in jellies, jams, brandy, liqueurs, conserves and vinegar, as traditional bread flour extender, diuretic, anti-inflammatory, anti-diarrhoeal, vasodilatory agent and vitamin source, while the leaves were used as antidiarrheal ingredients (Räty *et al.*, 2016). Moreover, in folk medicine the fruits and inflorescences of *S. aria* (L.) Crantz have been used as a diuretic, laxative and emmenagogue remedy for treating painful menstruation, kidney disorders and constipation. The berries of *S. intermedia* (subg. *Aria)* have been added to bread in Estonia (Kalle & Sõukand, 2012). Among this big subg., only two cvs *Aria rupicola* (Syme) Mezhenskyj and *S. intermedia,* grow naturally in the western part of Estonia and on island Saaremaa. However, a cultivar *S. aucuparia* L., is the most common plant in Estonia (Luuk, 2021).

The largest subg. *Sorbus* includes about 50 species that grow in the northern hemisphere in Europe and North America, but also in Asia (Robertson *et al.*, 1991b). The subg. *Sorbus*, commonly noted as mountain ash (Amur or European mountain ash), rowan or quick beam, has hairless or thinly hairy leaves. Asian people (Chinese, Japanese, Koreans, Tibetans) have used all anatomical parts of various *Sorbus* species, such as fruit, stems, bark and leaves as traditional remedies. For instance, the leaves of *S. tianschanica* Rupr. have been used to treat asthma, dyspnoea, tuberculosis, ventricular myocytes, and gastritis (Ayupbek *et al.*, 2012), while the stems and bark of *S. commixta* Hedl. have been used to treat inflammatory diseases and arthritis, and are used as vasorelaxant, hypoglycaemic, antitussive and

tonic agents (Bhatt *et al*., 2009; Moon *et al*., 2018). Moreover, when the berries of *S*. *cashmiriana* Hedl. have been used to cure scurvy, then the bark preparation has been used to treat nausea and heart diseases in traditional medicine (Khan *et al*., 2015). The fruits, stems and bark of another Asian species, *S*. *pohuashanensis* have been widely used in traditional Chinese medicine for treating chronic tracheitis, oedema and tuberculosis (Li *et al*., 2012). Due to the long-term effect in traditional medicine, the content of bioactive compounds and potential uses of rowan anatomical parts as nutraceuticals are being studied.

From the other *Sorbus* species both, the leaves and the bark of *S*. *decora* (Sarg.) C.K.Schneid., as well as the bark of *S*. *americana* Marshall, have been used for treating diabetes and are known as an antidiabetic medicine (Bailie *et al*., 2016). The other applications of *S*. *americana* Marshall include antitussive, vaso-relaxant and tonic agent (Mccune *et al*., 2002). The fruits of *S*. *sambucifolia* (Cham. et Schltdl.) M.Roem. have been used in drinks and foods (beverages, jams, jellies, dried fruit flour, etc.), as well as for medicinal purposes—in case of avitaminosis, arteriosclerosis and as antipyretic or diuretic agent. Native Americans used to eat fresh *S*. *scopulina* Greene berries; however, currently they are sometimes used in pies, preserves or wine-making (Mccune *et al*., 2002).

The fruit extracts of the most widely investigated rowan species *S*. *aucuparia* L. (European rowan), have traditionally been used as a good source of vitamins, an appetite-improving agents, mild laxative and diuretic medicine, as well as anti-inflammatory and vasodilatory agent (Gil-Izquierdo & Mellenthin, 2001; M. Olszewska, 2008). (Table 1 in **I**). In addition, the tea, syrup or alcoholic tincture of *S*. *aucuparia* L. fruits have traditionally been used to treat flu, infections, kidney disease and rheumatism (Fomenko *et al*., 2016).

2.1.2. Breeding of *S. aucuparia* L. in Russia

The wider application of *S*. *aucuparia* L. fruits for foods has been limited due to the astringent taste. Therefore, most of rowan trees were grown and bred mainly for decorative purposes in Europe and elsewhere. However, in the 19th century the sweeter clones of *S*. *aucuparia* L. from the Sudety Mountains (the Moravian Mountain Ash) were chosen for breeding. The cultivars of Russian origin, such as 'Krasnaja', 'Solnechnaja', 'Sahharnaja', 'Oranzevaja', 'Kubovaja' and many others

originate from the village of Nevezhino in Russia. These cultivars can be found in the Siberian regions of Russia as well as in northern European countries and they are more frost-resistant in addition to their yields being usually higher than that of the varieties of Moravian Mountain Ash (Jurikova *et al.*, 2014).

At the beginning of the 20th century Russian plant breeder Mitchurin and his co-workers used interspecific hybridisation to breed the crosses of *S. aucuparia* L. with *Malus*, *Aronia, Pyrus*, and *Crataegus* species (Sokolov *et al.*, 2015). For instance, in 1918, the rowan hybrid cv. 'Burka' was obtained by crossing *S. aria* (L.) Crantz, *Aronia arbutifolia* and *S. aucuparia* L. Seven years later (1925), Mitchurin crossed *S. aucuparia L.* and *Crataegus sanguinea* resulting in a new cv. 'Granatnaja'. Next year (1926), the red large-fruited cv. 'Alaja Krupnaja' was created by crossing the rowanberry (*S. aucuparia* L.) and pear (*Pyrus* sp.) with Moravian rowan (*S. aucuparia* L. var. *moravica*). A couple of years later, Mitchurin`s employee Tikhonova succeeded in creating the cvs 'Krasavitsa' and 'Rubinovaja' by crossing the rowan (*S. aucuparia* L.) and pear (*Pyrus sp.*). In 1949, another Russian breeder Yakovlev crossed Alpine rowan (*S. alpina* (Wild.) Scheid.) and German black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) and obtained sweet-fruited rowan cv. 'Likernaja' (Sokolov *et al.*, 2015).

Considering the significant variety of sweet rowanberry cultivars, the interest in them as potential sources of phytochemicals has recently increased. The sweet rowanberry hybrids are usually bigger and more palatable than the wild rowanberries, because they obtain the traits from both parents (initial forms) during crossing. Moreover, the hybrid cvs also possess higher yields and higher total polyphenolic and flavonoid contents, which in turn is related to higher antioxidant capacity (Jurikova *et al.*, 2014). Couple of studies have demonstrated the remarkable antioxidant characteristics of *S. aucuparia* L., especially the hybrid cvs, compared to some other species in the big genus Sorbus (Mikulic-Petkovsek *et al.*, 2017a; Raudonis *et al.*, 2014; Zymone *et al.*, 2018).

For total valorization of fruits, it is important to understand the benefits of by-products, such as their composition and antioxidant potential. Recently, the black chokeberry pomace was reported as a rich source of bioactive compounds such as anthocyanins and phenolic acids (Tamkute *et al.*, 2021). The pomace of genus *Crataegus* or hawthorn and *Pyrus* species or pear also possess a high antioxidant capacity (Hong *et al.*, 2021; Jiang *et al.*, 2020). The varieties of chokeberry, hawthorn, as well as

the pear were used in the interspecific hybridization with rowanberries for obtaining the cvs with better characteristics like high antioxidant potential and polyphenolic content.

2.1.3. Nutritional composition of anatomical parts of rowan species

The benefits of rowan species are related to the significant amounts of phytochemicals present such as phenolic acids, carotenoids, vitamins. The fruit of rowans also contain sugar alcohols, namely xylitol and sorbitol (Lee, 2015), which are suitable as a sweetener for people suffering from diabetes as these compounds metabolize slowly in the human body (Termentzi, Alexiou, *et al*., 2008). Regarding the sugars, such as fructose, glucose and sucrose have been identified in rowan species and the major sugar in rowanberries is glucose. The highest content of sugars was found 217.5 g/kg in *S*. *torminalis* (L.) Crantz (Mikulic-Petkovsek *et al*., 2017b). Some fruit species, such as apricot, peach or plums, which have been used for jams, contain less sugars than some rowan genotypes (H. Bae *et al*., 2014).

Among the organic acids, malic acid is the most dominant acid in rowans (from 60% to 88%) (Mikulic-Petkovsek *et al*., 2017b). The other organic acids in rowan species are citric and tartaric acid (from 7% to 39%) and shikimic and fumaric acids (less than 0.1%)

Sorbus species also contain a considerable amount of minerals, such as iron, potassium, and magnesium. For instance, a considerable amount of 8 essential minerals was detected in wild rowanberries: potassium, 154; phosphorus, 12.3; calcium, 29.9; magnesium, 27.84; iron, 2.42; copper, 0.294; zinc, 0.861; and manganese 0.503 mg/100 g (Aslantas *et al.,* 2007). Moreover, the tree bark of *Cormus domestica* (L.) Spach has been found to be as a good source of Ca, Zn, Fe, while the seeds were rich in K, Mg, Fe and Zn (Majić *et al*., 2015). The rowanberries can contain higher amounts of ascorbic acid 88.57 mg/L (Poyrazoğlu, 2004) compared to medium-sized oranges 70 mg/L (Moore *et al*., 2019).

Tocopherols are important fat-soluble vitamins in berries. The mean concentrations of α-tocopherol, δ-tocopherol, and γ-tocopherol in *Sorbus aria* (L.) Crantz and *S*. *aucuparia* L. were reported 2.82, 0.11, 2.01 μg/g dw and 4.89, 0.58, 1.71 μg/g dw, respectively (Yang *et al*., 2011).

Moreover, even 3.34 μg/g dw of α–tocopherol was detected in *S. aucuparia* L. fruit, however the content of γ-tocopherol was remarkably lower, 0.25 μg/g dw (Klavins *et al.*, 2016).

In nature, β-carotene, a precursor (inactive form) of vitamin A, is a strongly coloured red-orange pigment, which is abundant in some plants and fruits, including *Sorbus* species. For instance, *S. aucuparia* L. contains 2.5 mg of total carotenoids per 100 g (Berna *et al.*, 2012). The average daily intake of β-carotene for adults is in the range of 2–7 mg.

Plant oils are important as food ingredients and as a source of essential fatty acids for human nutrition. The sum of linoleic and oleic acids have been found to exceed 90% of the total fatty acids in seed oils of *S. aucuparia* L. (Presler *et al.*, 2012). The tocopherols and ascorbic acid as well as the remarkable amount of carotenoids also make a significant contribution to antioxidant capacity of rowanberries and their pomace (Šavikin *et al.*, 2017).

2.1.4. Promising applications of *Sorbus* species in nutraceuticals and pharmaceuticals

It is obvious that among phytochemicals and other nutrients, polyphenolic compounds, especially phenolic acids and flavonoids, can be considered as the most beneficial health promoting constituents detected in several anatomical parts of *Sorbus* species. The polyphenolic flavonoids, which influence the flavour and colour of plants, have demonstrated different health benefits to consumers. These benefits include antidiabetic, anti-hyperlipidemic, antiviral, antifungal, antitumoral effects, as well as vasoprotective, neuroprotective, hepatoprotective activities (Cheon *et al.*, 2017; Fomenko *et al.*, 2016; Gu *et al.*, 2016; Raspé *et al.*, 2000; Razina *et al.*, 2016; Termentzi, Alexiou, *et al.,* 2008; Termentzi, Kefalas, *et al.*, 2008; Yu *et al.*, 2011; Zymone *et al.*, 2018).

The most famous Asian rowan species are *S. commixta, S. tianschanica* Rupr. and *S. cashmiriana* Hedl.*,* as their bark, twigs, leaves or fruit extracts have demonstrated UV- protective, anti-inflammatory and anti-cancer agents (J. Bae *et al.*, 2007; Lee *et al.,* 2017; Yu *et al.*, 2011).

With regard to the other *Sorbus* species, the branch extract of *S. alnifolia* (Siebold et Zucc.) K. Koch (subg. *Micromeles*) has shown to have potential

in the treatment of Parkinson's disease (Cheon *et al*., 2017). *S*. *torminalis* (L.) Crantz (subg. *Torminaria*) extracts have inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* in the *in vitro* trials (Mrkonjić *et al*., 2017).

There is substantial evidence for the antidiabetic effects of *Sorbus* species. For instance*, Cormus domestica* (L.) Spach fruits can reduce the complications of early stage type 2 diabetes (T2D) (Termentzi, Alexiou, *et al*., 2008). Species of Subg. *Aria*, especially *S*. *norvegica* Hedl., have given good results in T2D drug trials (Broholm *et al*., 2019). Moreover, the fruits of *S*. *aucuparia* L. have demonstrated the ability to maintain postprandial glycemic control in T2D (Boath *et al*., 2012).

Finnish researchers have found that polyphenols from two hybrid cvs of *S*. *aucuparia* L., 'Zoltaja' and 'Granatnaja' have delayed pathogenic *E. coli* growth and the phenolic extracts of wild rowanberries and hybrid cv 'Burka' have inhibited the hemagglutination of *E. coli* HB101 (pRR7), which expresses the M hemagglutinin (Kylli *et al*., 2010). Water and methanol extracts of fruits *S*. *aucuparia* L. were also effective in the inhibition of acetylcholinesterase (AChE) and exhibited *in vitro* cytotoxicity in SRB assay, using tumour HeLa, MCF7 and HT-29 and healthy MRC-5 cell lines (Mrkonjić *et al*., 2017). Moreover, these extracts possessed the high mitogenic activity expressed as the stimulating effect on hamster lymphocyte proliferation, but not yet on human.

2.2. The content of antioxidant polyphenols in different anatomical parts of rowan plants

Polyphenols, which are a group of secondary metabolites in plant-derived food, are considerable antioxidants in the human diet. They consist of various compounds, such as phenolic acids, flavonoids, stilbenes, coumarins and lignans and their derivatives. The flavonoids in turn are divided to many subgroups, such as anthocyanins, flavanols, flavanones, flavones, flavonoids and isoflavonoids (Han *et al*., 2007).

During recent decade, several authors have discovered *Sorbus* species as interesting plants for investigation in terms of high content of phytochemicals. The main phenolic constituents in rowanberries are caffeoylquinic acids, such as neochlorogenic (5-O-caffeoylquinic and chlorogenic (3-O-caffeoylquinic, 3-CQA) acids, which constitute 56-80% of total phenolics (Kylli *et al*., 2010; Šavikin *et al*., 2017) (Fig.2 and 3).

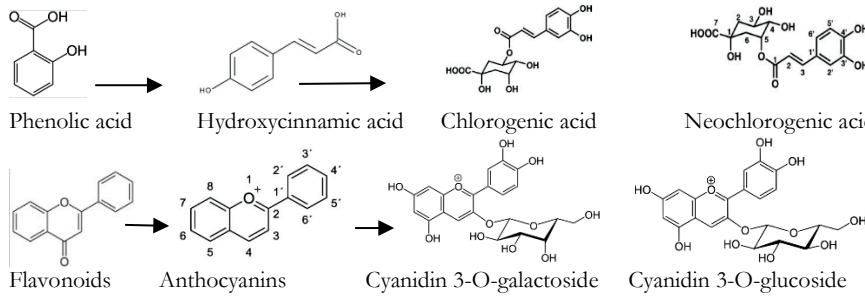


Figure 2. The most dominant phenolic acids and flavonoids in rowan species

Moreover, the statistically significant correlation has been found between caffeoylquinic acids and antioxidant activity (Kähkönen *et al.*, 2001). The highest content of caffeoylquinic acids have been analyzed in wild rowanberries (Kylli *et al.*, 2010). The inflorescences and leaves have higher TPC values as well as higher chlorogenic acid content than these in the fruits (M. A. Olszewska *et al.*, 2010; Šavikin *et al.*, 2017).

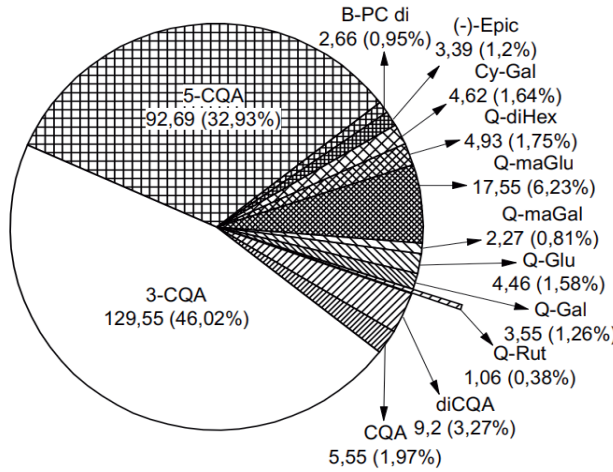


Figure 3. The composition of polyphenols in rowanberries (Tian *et al.*, 2017).
3-O-caffeoylquinic acid (3-CQA), 5-O-caffeoylquinic acid (CQA), dicaffeoylquinic acid (diCQA), 3-O-rutinoside (Q-Rut), quercetin 3-O-galactoside (Q-Gal), 3-O-glucoside (Q-Glu), quercetin 3-O-(malonyl)-galactoside (Q- maGal), quercetin 3-O-(malonyl)-glucoside (Q- maGlu), quercetin-dihexoside (Q-diHex), cyanidin 3-O-galactoside (Cy-Gal), (-)-Epicatechin ((-)-Epic), B-type procyanidin dimers (B-PC di)

The flavonoids are the other major group of polyphenolic secondary metabolites in *Sorbus* species. They are the most important plant pigments, producing yellow or red pigmentation. Anthocyanins, such as

cyanidin 3-galactoside, cyanidin 3-glucoside, and cyanidin 3-arabinoside, are a class of water-soluble flavonoids in rowanberries (Hukkanen *et al.*, 2006). Flavonols are the colourless group of polyphenols belonging to the flavonoid family. The main flavonols identified in rowanberries are quercetin and kaempferol glycosides (Boath *et al.*, 2012). The flavonols and anthocyanins together with the main polyphenolic compounds, caffeoylquinic acids, are responsible for antioxidant properties of *Sorbus* species (Kylli *et al.*, 2010; M. A. Olszewska *et al.*, 2010).

Among the other phenolic acids, protocatechuic acid was found in the fruits, leaves and bark of some *Sorbus* species as well as in the extracts of inflorescences also in the leaves and in the fruit pulp (Kim *et al.*, 2010; M. Olszewska *et al.*, 2012; Rutkowska *et al.*, 2019; Termentzi, Kefalas, *et al.*, 2008). Moreover, the ferulic acid was detected in the leaves of some species (Ekin *et al.*, 2016; Pasko, 2012). Some other phenolic acids, such as cinnamic, vanillic, caffeic, p-coumaric and benzoic acids were found only in traces in the various *Sorbus* species (Isaikina *et al.*, 2018; Kim *et al.*, 2010; Mikulic-Petkovsek *et al.*, 2017b; M. A. Olszewska *et al.*, 2012).

2.3. Technologies for the extraction of phytochemicals from rowanberry pomace

Juicing of fruits produces a significant amount of press residue or pomace, which can pollute the environment if it`s discarded (Venskutonis, 2020a). However, this by-product could be a potential source of several phytochemicals for food or non-food applications The investigations have proved that the significant amount of polyphenols still remain in different parts of the pomace, such as seeds, skins and pulp, 60-70%, 28-35% and 10%, respectively (De Ancos *et al.*, 2015). In addition, berry pomace also contains a significant amount of dietary fiber, carbohydrates and protein, as well as unsaturated fatty acids, such as oleic (18:1), linoleic (18:2), and α-linolenic (18:3) acids, which can be used for the benefit of human health. The content of particular fruit pomace depends on the particular berry fruits.

Recently, the innovative extraction techniques, such as the ultrasound- (UAE) and microwave-assisted (MAE) as well as pressurized liquid (PLE) and supercritical fluid (*e.g.*, SFE-CO_2) extraction have been used for extraction of phytochemicals from plant materials. These extraction techniques enable decreasing the amount of solvents and the extraction

time, compared to the conventional methods (Vilkhu *et al.*, 2008). In addition to extraction technology, the pre-treatment of the starting material as well as the suitable extraction solvents are also essential for achieving the maximum extraction rate and yield (Pasquel Reátegui *et al.,* 2014). In most cases, the extraction solvents, which are generally recognised as safe (GRAS), replace the poisonous organic solvents.

In case of UAE, the ultrasound creates the micro bubbles in the liquid, which will be expanded and imploded due to the acoustic cavitation. Cavitation is the phenomena, where the boiling of liquid is caused by the reduction of pressure at ambient temperature (Pingret D *et al.*, 2017). The cavitation causes the cell disruption and the effective mass transfer, therefore increasing the efficiency of extraction (Zahari *et al.*, 2020). The scanning electron microscope (SEM) images have demonstrated remarkable cell wall disruptions during the UAE procedure, compared to conventional extraction (Kashyap *et al.*, 2022). Microwaves are electromagnetic waves which energy is created by a non-ionizing radiation. Microwaves are composed of electrical field and magnetic field, which are perpendicular to each other (Letellier & Budzinski, 1999). The microwave energy can be changed to thermal energy by ionic conduction and dipole rotation in solvent and in the sample caused by applied electromagnetic field. The migration of ions enhances the possibilities of solvent penetration into the solid matrix and promotes the dissolution of aimed compounds (Letellier & Budzinski, 1999). MAE has several advantages compared to the conventional extraction, such as decrease of extraction time, energy and solvent consumption, as well as the higher efficiency of extraction of bioactive compounds (Davis *et al.*, 2021; Ekezie *et al.*, 2017; Routray & Orsat, 2012).

PLE is an extraction method, where the extraction solvent at elevated temperature and pressure is applied to the plant material, in order to decrease the viscosity and the surface tension of solvent, therefore enhancing the mass transfer rate and increasing the extraction yield (Andrade *et al.*, 2021). Under elevated temperature and pressure, the solvents are below their critical point and keep the liquid phase during the procedure, enabling the penetration of the solvent easier into the every matrix being extracted (Andrade *et al.*, 2021). In this case, when the pressurised hot water is used as the extraction solvent, the method is called 'Pressurized Hot Water Extraction' (PHWE) or 'Sub-critical Water Extraction' (Mustafa & Turner, 2011). It has been observed

that the PLE extracts could possess extensive applications as disease preventive functional foods and nutraceuticals due to the high contents of antiproliferative and antioxidant substances (Pukalskienė *et al.*, 2021). The comparative study of the effect of different solvents, such as acetone, ethanol and water on wild rowanberry pomace by using pressurized liquid extraction (PLE) method was thereafter conducted, achieving the highest concentration of chlorogenic acid (3970 μg/g extract) with ethanol extract (Bobinaitė *et al.*, 2020). In addition, acetone extract of rowanberry pomace was the strongest antimicrobial agent, as well both acetone and water extracts exhibited higher cytotoxic effect towards cell viability assays (SRB and MTT) using Caco-2 cells (Bobinaitė R *et al.*, 2020).

The PLE is often combined with some other extraction methods, such as UAE, MAE or SFE-CO_2 extraction, to increase the yield of desired phytochemicals. Recently, wild rowanberry pomace was extracted by applying consecutive PLE and supercritical carbon dioxide (SFE -CO_2) achieving the total extraction yield 33.1% of polyphenol-rich extract as well as the recovery of total carotenoids up to 49.7% (Bobinaite *et al.*, 2020).

The SFE technology usually applies CO_2 as an environmentally friendly, non-toxic and non-flammable solvent, which at particular conditions, such as 31.1 °C and 73 atmospheres, behaves as a liquid and a gas simultaneously. At these conditions, CO_2 diffuses perfectly like gas, but it has also the inherent density of liquid. Moreover, the dissolving ability of CO_2 is strongly dependent on the pressure and temperature, which both are easily adjustable (Herrero *et al.*, 2010).

In addition to polar polyphenolic compounds, berry pomace contains a considerable amount of lipophilic compounds including polyunsaturated fatty acids (PUFAs), triacylglycerols (TAGs), tocopherols, phytosterols, carotenoids and volatile aroma compounds (Venskutonis, 2020a). Many authors have applied SFE-CO_2 for recovering mainly lipophilic components from the seeds and pomace of several fruits (Basegmez *et al.*, 2017; Grunovaite *et al.*, 2016; Kitryte *et al.*, 2020; Kryževičiute *et al.,* 2016; Yang *et al.*, 2011). However, in some cases the co-solvents, such as ethanol or water have been applied to extract the polar components together with non-polar lipids and to achieve higher yields.

The process conditions are optimized for each technique and specific plant material before being used on a larger scale. The effective multivariate techniques, such as response surface methodology (RSM), enable optimizing more than one variable simultaneously. For final optimization, three multilevel designs, such as Box–Behnken design (BBD), central composite design (CCD) and Doehlert matrix (DM) have been routinely applied (Zolgharnein *et al.*, 2013). Recently, two groups of researchers optimized the parameters by applying central composite design (CCD) and response surface methodology (RSM) in order to obtain the highest lipophilic extract yield of rowanberry pomace by SFE-CO_2. One group found that the optimum conditions for rowanberry pomace SFE-CO2 extraction were 325 atm, 85 °C, and 72 min of extraction time (Ivakhnov *et al.*, 2019). In this case the yield of the oil reached to 9.02 wt%. The other group achieved the highest extraction yield of 4.80% by using 60 °C, 45 MPa and 180 min of extraction time, and the results of experiments demonstrated that the changes in SFE-CO_2 parameters remarkably influenced the content of carotenoids in the extracts (Bobinaite *et al.*, 2020). Considering the low recovery of carotenoids (49.7%) and the low extract yield compared to the hexane extraction, the co-solvent addition and the fractionation was planned.

3. HYPOTHESES AND AIMS OF THE STUDY

The main aim of the present study was to find out, among 16 sweet rowanberry cultivars and wild rowanberry, the most promising ones with the highest total polyphenolic content (TPC) and antioxidant capacity, and valorize their juice pressing by-products by converting them into high-value functional ingredients.

Hypotheses:

- A significant part of phytochemicals with high antioxidant potential and polyphenolic content remain in the rowanberry pomace fraction.
- The rowanberry pomace-based ingredients can be developed into functional ingredients for food applications

The specific aims of the present study were:

1) To provide a comprehensive review of existing knowledge on various aspects of rowan (*Sorbus* spp.) grown all over the world, such as traditional applications, chemical composition, especially the specific phenolic composition and antioxidant activity of different plant parts; identification of promising trends in increasing the value of rowanberry crops **(I)**

2) To characterize the fruit, juice, and pomace of 16 sweet rowanberry cultivars and wild rowanberry the most productive rowanberry (*Sorbus aucuparia* L.) cultivars grown in Estonia for their antioxidant properties and polyphenolic composition. As a result, the most promising cultivars were selected for further studies (**II**)

3) To develop a rowanberry biorefining scheme for applying rowanberry pomace-based ingredients in minced meat product (meatballs) in order to delay oxidation processes **(III)**

4. MATERIAL AND METHODS

4.1. Antioxidants characterization of the fruit, juice, and pomace of sweet rowanberry (*Sorbus aucuparia* L.) (II)

4.1.1. Sample preparation

Antioxidants characterization of the fruit, juice and pomace of 16 sweet rowanberry cultivars and wild rowanberry was carried out using the fruit from the experimental orchard of Polli Horticultural Research Centre, Estonia (58°21'N, 26°40'E, 68 m above sea level) (Table 1 in **II**). The sweet rowanberry cultivars studied were the following: 'Burka', 'Alaja Krupnaja', 'Granatnaja', 'Kubovaja', 'Rosina', 'Rubinovaja', 'Angri', 'Bussinka', 'Likernaja', 'Moravica', 'Oranzhevaja', 'Krasnaja', 'Sahharnaja', 'Solnechnaja', 'Rossica', 'Vefed'. The full pedigree of these cvs is in Table 1 in paper **II**.

4.1.2. Measurements and analyses

The visually ripe fruit of sweet rowanberry cvs (approximately 5 kg each) were collected for analyses and for storage at the end of August and beginning of September in years 2019 (**II**) and 2021 (**III**) considering the average ripeness of cvs. The sample was divided into two parts. Half of the sample of each cv was stored at −20 °C. The second half of the sample was used for extraction the juice with the low-speed juicer Smeg SJF01CREU (Smeg S.p.A, Guastalla, Italy). The rowan fruit, juice, and pomace were freeze-dried in an Advantage Plus Benchtop Freeze Dryer (SP Industries, Warminster, PA, USA) and the pomace samples were ground in a Retsch Mixer Mill M 400 (Haan, Germany) (**II**). Lyophilized juice samples were dissolved in methanol (MeOH, 1 w/v) and treated for 15 min in the ultrasound bath. The centrifuged solutions were used for measurements with the QUENCHER procedure Serpen *et al.* (2007) for evaluating the antioxidant capacity of freeze-dried fruit and pomace. For preparation of the stock mixture, the lyophilized pomace was mixed with microcrystalline cellulose at a ratio of 1:1 (w/w). To obtain the concentrations in the range of 1–40 μg/mg, couple of dilutions of stock mixture with microcrystalline cellulose were prepared and according to this 10 mg of freeze-dried pomace were used in further experiments.

Total phenolic content (TPC) was determined by the Folin-Ciocalteau phenol reagent method (Singleton *et al.*, 1999). The absorbance was read at 765 nm wavelength (**II**, **III**).

In 2,2-diphenyl-1-picrylhydrazyl (DPPH•) scavenging capacity determination according to Brand-Williams *et al.* (1995), the juice and fruits extracts (7.5 μL, 0.1%) were mixed in a FLUOstar Omega 96 well microplate reader with 300 μL of DPPH• and the pomace or cellulose as a blank sample were mixed with-500 μL of MeOH and 1000 μL of working DPPH• solution. The absorbance of samples was measured at 515 nm comparing it with a blank.

In 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+) assay according to Re *et al.* (1999), the samples of juice and fruit extracts (3 μL, 0.1%) were mixed with 300 μL ABTS•+ solution and pomace or cellulose as blank were mixed with 25 μL of MeOH and 1500 μL of working ABTS•+ solution in the microplate wells of FLUOstar Omega reader, measuring the absorbance at 734 nm against phosphate-buffered saline solution as a dilution blank.

In oxygen radical absorbance capacity (ORAC) assay by Prior *et al.* (2003), fluorescein was used as a fluorescent probe in 75 mM phosphate buffer (pH 7.4). The addition of antioxidant substances originated from rowanberry fractions produced a fluorescent signal, which could reflect the antioxidant capacity. The 485 nm excitation and 520 nm emission filters were used. The antioxidant capacity in all assays is expressed as μM of Trolox equivalent (TE) per gram of dry weight sample.

For the analysis of individual phenolic compounds, an ultra-high-performance liquid chromatography (UHPLC) was used, mixing 1 g of fresh fruit, juice, or pomace in 10 mL of 50% ethanol acidified with 1% of HCl. The identification of individual phenolic compounds was conducted by comparing their retention times, UV spectra, and parent and daughter ion masses (m/z) with the same data of the reference compounds. All the samples were analyzed in triplicate, and the results were expressed as milligrams per gram of dry weight.

4.2. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs (III)

4.2.1. Experimental design (III)

Sweet rowanberry cvs 'Likernaja' (the hybrid of *S. aucuparia* L. × *Aronia melanocarpa*), 'Solnechnaja' (the seedling of *S. aucuparia* L.) and wild rowanberry were harvested in autumn 2021 from the experimental orchard of Polli Horticultural Research Centre, Estonia, (South Estonia, 58°07´44.5´´N, 25°32´16.8´´E). The pomace of rowanberries was treated as it was described in chapter 4.1. All three pomace samples were mixed and ground in Retsch cutting mill Retsch SM 300, (Retsch GmbH, Haan, Germany) with sieve holes diameter of 5 mm and defatted by supercritical CO_2 by Separex 5 (Champigneulles, France) at 40 MPa pressure, 40 °C according to Tamkute *et al.* (2021) to obtain the 1st ingredient (AC) for meatballs.

AC was further extracted with 1:1 (v/v) ethanol/water at solid/liquid ratio of 1:10 (w/v) using microwave-assisted extraction (MAE) for 15 minutes at power 300 W. After the extraction, the extract was filtered. The EtOH part of the supernatant was dried in a rotary evaporator, and the remaining water was freeze-dried in a VirTis Advantage Plus Benchtop Freeze Dryer Model XL-70 (SP Industries, Warminster, PA, USA). The dried extract was stored in a sealed package in a freezer (-20 °C) as the 2nd ingredient (E) for meatballs. The extraction residue was freeze-dried and stored as the 3rd ingredient (R) in the grip seal polythene bag at room temperature (Fig.4).

In current study, the minced pork contained 67.43% moisture, 18.49% protein, 13.85% fat, and 0.96% ash. The meatball mixture was mixed with 11% water and 1% salt and with different concentrations (1-5%) of rowanberry pomace-based ingredient. The meatballs with different concentrations of ingredients were sensory evaluated. The best of each group was selected for further tests, while the meatballs without any ingredients were the reference samples.

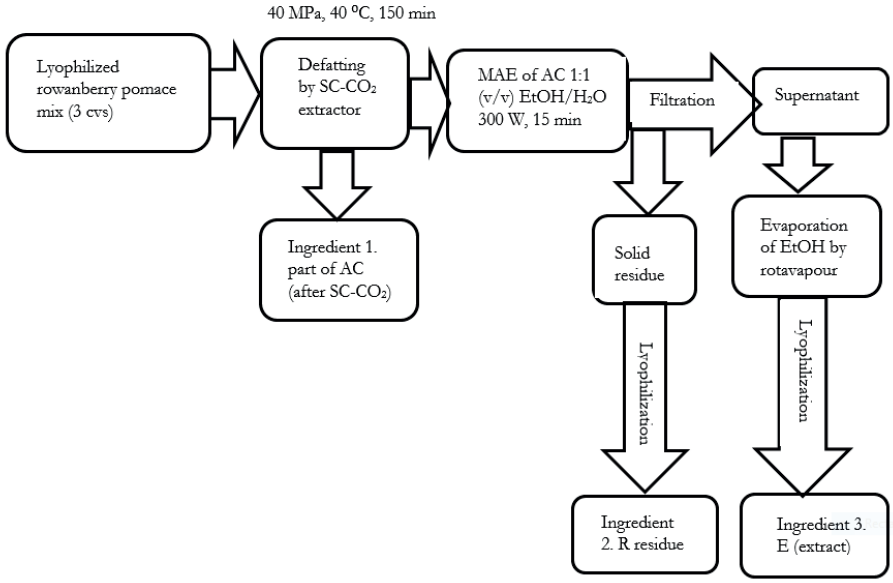


Figure 4. Preparation of rowanberry ingredients for pork meatballs

After weighing, the meatballs were cooked at 145 °C in the oven Inoxtrend E1CUA-107E (Santa Lucia di Piave, Italy) for 15 minutes. The cooled meatballs were weighed and packed under a modified atmosphere consisting of 70% N_2 and 30% CO_2. To understand the effect of rowan ingredients on the physicochemical parameters of pork meatballs during cold storage (+4 °C), the time points of day 0 and 5 were chosen for analyzing.

4.2.2. Measurements and analyses (III)

The sensory assessment of cooked meatballs was conducted by nine randomly selected trained assessors from Estonian University of Life Sciences, Chair of Food Science and Technology. The fresh meatballs were warmed to 55-70 °C and cut in half. The sensory attributes used the hedonic 9-point scale to evaluate the odour, appearance, colour, taste, juiciness, and texture of the cooked meatballs.

The determined quality characteristics included the cooking loss, pH, water activity and colour measurements. The cooking loss was calculated as the weight difference between raw and cooked meatballs in percentages. The meatball samples (5 g) were homogenised with 50 mL of 0.1 M potassium chloride solution for pH measurements with Seven 2Go™

pH-meter (Mettler-Toledo AG Analytical, Schwerzenbach, Switzerland). The water activity (aw) was analysed by the water activity analyser (Aqua Lab, Model Series 3 TE, Decagon Devices, Inc., Washington, DC, USA). The colour measurements were conducted by X-Rite 964 spectrophotometer (X-Rite, Grand Rapids, MI, USA) measuring three replicates of each freshly cut meatball sample from different places. The data were expressed numerically by CIE (International Commission on Illumination) Lab system values (Mokrzycki & Tatol, 2012).

The chemical analyses included fat (EVS-ISO 2446:2001, Gerber method), moisture (EVS-ISO 1442:1999), protein (EVS-ISO 937:1978, Kjeldahl method), and ash content (ISO 936:1999).Determination of total phenolic content (TPC) by (Folin & Ciocalteu, 1927) was conducted for preliminary screening of rowan fruit pomace based ingredients AC, R and E. In addition, *in vitro* (DPPH•) radical scavenging activity by Brand-Williams *et al.*(1995) with modifications was determined for meatballs with 2% of AC, 2% of R and 1% of E. The absorbance values of the samples were ascertained in four replicates at 760 nm and 515 nm, for TPC and DPPH• assays, respectively, using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan).

The untargeted metabolomics was used for evaluation of the phytochemical profile and storage effects (day 0, 4 and 14) on meatballs with 3 different rowanberry ingredients. For metabolomics, the procedure of Pateiro *et al.* (2018) with modifications was used. Briefly, 1g of lyophilized pork meatballs were extracted with 10 mL of an 80% aqueous methanol (v/v) solution (both LC-MS grade, VWR, Milan, Italy) with 0.1% (v/v) formic acid. Subsequently, the mixture was treated with Ultra-turrax (Ika T10, Staufen, Germany) for 5 min, centrifuged (Eppendorf 5810R, Hamburg, Germany) at 7800 *x g* for 15 min at 4 °C and filtered with 0.22 μm cellulose syringe filters into amber vials for instrumental analysis.

For the untargeted profiling analysis, the high-resolution mass spectrometry (HRMS) based on a Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) coupled to a Vanquish ultra-high-pressure liquid chromatography (UHPLC) pump, equipped with heated electrospray ionization (HESI)-II probe (Thermo Scientific, USA) was used.

The chromatographic separation was conducted under a gradient of acetonitrile in water (from 6% to 94% in 35 min) as mobile phase, with 0.1% formic acid as a phase modifier, using BEH C18 (2.1x100 mm, 1.7 μm) maintaining the analytical column at 35°C. The injection volume was 6 μL and elution flow rate was 200 μL/min. Full scan MS analysis was performed under the positive ionization mode and with a nominal mass resolution of 70,000 FWHM at m/z 200. The injection sequence was randomized, with 3 replicates for each sample. Quality control (QC) samples (prepared by pooling same aliquots of each sample) were acquired in a data-dependent (TOP N = 3) MS/MS mode, and the Top N ions were selected for fragmentation under stepped (10, 20, 40 eV) Normalized Collisional Energy. The HESI parameters were previously optimized by (Rocchetti *et al.*, 2021). The raw spectral data were processed using MS-DIAL software (version 4.80) for post-acquisition and data filtering procedures (Tsugawa *et al.*, 2015). The MS-DIAL parameters were adapted from previously published works on LC-MS untargeted metabolomics-based analysis (Rocchetti *et al.*, 2021). The mass features were searched in the mass range of 80-1200 *m/z*, having a minimum peak height of 10,000 cps. Accurate mass tolerance for peak centroiding was 0.05 Da for MS and 0.1 Da for MS/MS analysis. Retention time information was excluded from the calculation of the total identification score. The MS and MS/MS tolerance for identification was set to 0.05 Da and 0.1 Da, respectively. The identification step was based on mass accuracy, isotopic pattern (i.e., isotopic distribution, space, and abundance) and spectral matching. The total identification cut-off score was set to 50%, retaining the most common HESI+ adducts. Annotation of meat metabolites was achieved against the comprehensive database known as FooDB (http://foodb.ca/). Furthermore, the software MS-Finder Tsugawa *et al.* (2016) was used for in-silico fragmentation of the not annotated mass compounds, using the FooDB and Lipid Maps libraries, thus working according to a level 2 of confidence in annotation (i.e., putatively annotated compounds and structural confirmation according to spectral matching) (Salek *et al.*, 2013). Only the compounds having an in-silico prediction score higher than 5 were retained.

4.3. Statistical data analysis

The MS Excel and one-way analysis of variance (ANOVA) at p value < 0.05 were used to calculate the mean values and standard deviations (SD) of radical scavenging capacity results and total phenolic contents

and correlation coefficients (R^2) between two RSCy assays and the polyphenolic groups. A principal component analysis (PCA) of eight major phenolic compounds was used for the rowanberry fruit, juice, and pomace samples (Figure 4 in **II**). The plots (a, b, c) demonstrated the differences between the dark red hybrid cvs group (blue) and the other sweet rowanberry cvs orange-coloured group (red)(**II**).

The statistical package R 4.2.0 was applied for statistical analysis (Minato Nakazawa, 2022) of assessors` panel sensory scores and sensory score results visualization. The Linear Mixed-Effects Model (GLMM) was used to study the effects of variants, the effect of three replications and the storage period on the pH, aw and colour characteristics of the samples as well as the measurements of cooking loss, moisture, and protein and ash contents on the day 0. The Emmeans Searle *et al.* (1980) package was applied for the pairwise comparison of groups and the model-assessed results were presented as least-square means (**III**). The multivariate statistical analyses dealing with metabolomics were done using two different software, Mass Profiler Professional (version B.12.06; from Agilent Technologies) and SIMCA (version 16; from Umetrics, Malmo, Sweden) for data processing and normalization and supervised modelling, respectively. In this regard, both unsupervised and supervised multivariate statistics were used based on hierarchical cluster analysis (HCA), Principal Component Analysis (PCA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA). The OPLS-DA models were built considering the storage period (i.e., 0, 4, and 14 days) under investigation, also recording the model validation parameters (goodness-of-fit R^2Y) and goodness-of-prediction Q^2Y). The VIP (i.e., variables importance in projection) selection method was then used to list the most relevant meat metabolites in prediction, considering only VIP markers characterized by values higher than 1. Finally, a Fold-Change (FC) analysis was done to check the direction and the intensity of variation of the marker compounds highlighted by the VIP selection method (**III**).

5. RESULTS

5.1. Antioxidants characterization of the fruit, juice, and pomace of sweet rowanberry (*Sorbus aucuparia* L.) (II)

5.1.1. Total phenolic content (II)

The TPC values of 16 sweet rowanberry cvs ranged between 2.53 and 15.05 mg GAE/g dw, 0.53 and 14.8 mg GAE/g dw, and 15.97 and 44.68 mg GAE/g dw for whole fruit, juice, and pomace fractions, respectively (Table 2 in **II**). The highest levels were found for all fractions of hybrid cvs 'Likernaja', 'Burka', 'Rubinovaja', and 'Granatnaja'. The highest TPC values were determined for pomace fractions of cvs 'Burka', 'Likernaja' and 'Rubinovaja', 44.68 mg GAE/g dw, 41 mg GAE/g dw and 41 mg GAE/g dw, respectively.

Compared to the mean values of fruit and juice, pomace fraction had the highest mean value of TPC (Figure 2a in **II**). The cvs 'Likernaja' and 'Burka' had also high TPC values in the fruit, 15.05 and 14.78 mg GAE/g dw, respectively, and in the juice 14.8 and 9.68 mg GAE/g dw, respectively. Among the other (non-hybrid) cvs, the remarkably high TPC values were found in the pomace fractions of cvs 'Moravica' and 'Solnechnaja' as well as the wild rowanberry, 29.32, 28.3 and 31.7 mg GAE/g, respectively.

5.1.2. Antioxidant capacity (II)

The DPPH• scavenging activity ranged from 15.1 to 177.5 μM TE/g dw, 6.03 to 125.6 μM TE/g dw, and 172.1 to 527.6 μM TE/g dw, for fruit, juice, and pomace, respectively (Table 2 in **II**). ABTS•+ scavenging activity values ranged between 666 and 1068 μM TE/g dw, 123.2 and 641.4 μM TE/g dw, and 179.9 and 584.2 μM TE/g dw for fruit, juice, and pomace, respectively. The ORAC assay values ranged from 239.1 to 456.5 μM TE/g dw, 19.7 to 443.7 μM TE/g dw, and 43.87 to 150.8 μM TE/g dw, for fruit, juice, and pomace, respectively. All the antioxidant capacity values of hybrid cvs 'Likernaja', 'Burka', 'Rubinovaja', and 'Granatnaja' were higher than average.

The pomace fractions of the hybrid cvs 'Likernaja', 'Burka' and 'Rubinovaja' had the highest values of DPPH• (527.55 μM TE/g dw), ABTS•+ (576.77 μM TE/g dw) and ORAC (150.75 μM TE/g dw), respectively. All fractions of cv 'Solnechnaja' had remarkably high ORAC values, as well as the DPPH• and ABTS•+ values were higher than the mean values of 17 pomace samples. While the rise of DPPH• mean values is in the direction: juice < fruit < pomace, then the means of ORAC and ABTS•+ values raise in the direction: pomace < juice < fruit (Figure 2 in **II**).

5.1.3. Identification and quantification of individual phenolic compounds in different fractions of sweet rowanberry cultivars (II)

The individual phenolic compounds were identified by comparing their retention times and mass spectra with the same data of the reference compounds (as described in chapter 4.1.2). The results of UHPLC-DAD-MS/MS analysis demonstrated that sweet rowanberry cvs are rich in chlorogenic and neochlorogenic acids, ranging between 1.07 and 4.59 mg/g dw and between 0.75 and 6.13 mg/g dw, respectively (Figure 3 and Table 3 in **II**). The highest content of neochlorogenic acid was found in the juice samples of hybrid cvs 'Likernaja', 'Burka', 'Granatnaja', and 'Rubinovaja', 6.12 mg/g dw, 3.93 mg/g dw, 3.50 mg/g dw and 3.04 mg/g dw (Table 3 in **II**).

Moreover, the highest chlorogenic acid contents were determined in the juice samples of sweet rowanberry cvs 'Sahharnaja', 'Bussinka', 'Angri', and wild rowanberry, 4.99 mg/g dw, 4.84 mg/g dw, 3.46 mg/g dw and 3.59 mg/g dw (Table 3 **in II**). The neochlorogenic acids and chlorogenic acids were the most dominant phenolic acids also in pomace samples (Figure 3 in **II**). The highest values of the chlorogenic acid content in the pomace samples were determined in wild rowanberry and sweet rowanberry cvs 'Bussinka' and 'Sahharnaja', at 4.79 mg/g dw, 3.64 mg/g dw, and 3.62 mg/g dw, respectively.

The highest content of anthocyanins (ACY)-s were found in rowanberry hybrids, such as cvs 'Burka', 'Likernaja', 'Granatnaja', and 'Rubinovaja', 7.27 mg/g dw, 6.33 mg/g dw, 3.20 mg/g dw, and 2.28 mg/g dw, respectively (Figure 3 and Table 3 in **II**). Cyanidin-3-galactoside (up to 91% for 'Rubinovaja') covered the major part of ACY-s in the fruit

and juice samples of rowanberry hybrids and cyanidin-3-arabinoside (up to 21–22% for 'Likernaja' and 'Burka') took the second place of ACY-s. However, the fruit and juice samples of the other rowanberry cvs contained less than 1 mg/g dw ACY-s. In the case of rowanberry pomace samples, cyanidin-3-glucoside covered the major part (up to 97%) of ACYs (Figure 3 in **II**). The highest total content of ACYs was found in the pomace samples of hybrid cvs 'Burka' and 'Likernaja', 'Rubinovaja' and 'Granatnaja'. The mean value of ACYs was up to 10 times lower in the pomace samples than in the fruit and juice samples. However, the mean value of flavanols in the juice and fruit samples was approximately 4.8 times lower than that in the pomace samples.

These comprehensive antioxidant capacity and polyphenolic content analyses helped to choose the cultivars for further studies. In addition, the cvs were selected according to their yield in years 2019 and 2021. Finally, hybrid cvs 'Likernaja' and 'Burka', as well as sweet rowanberry cvs 'Sahharnaja' and 'Solnechnaja', however the wild rowanberry stood out from others and could be used in further applied research.

5.1.4. The correlation between antioxidant assays and different polyphenolic groups (II)

The significant correlations were found between the ORAC, ABTS•+, and DPPH• scavenging values and the main phenolic groups in rowanberry fruit, juice, and pomace fractions (Table 4 in **II**).

In addition, relatively strong positive correlations were discovered between the TPC-s as well as ACY contents and all antioxidant assays of the pomace, fruit, and juice extracts ($0.49 < R^2 < 0.95$) and ($0.48 < i2 < 0.89$), respectively (Table 4 in **II**). The moderate correlations were found between ORAC, ABTS•+, as well as DPPH• scavenging values and FLAVO contents ($0.47 < R^2 < 0.66$), however, the correlation between DPPH• and FLAVO of fruit was weak ($R^2 = 0.28$) (Table 4 in II). There were no significant correlations found between radical scavenging activity values and the FLAVA contents in fruit, juice, and pomace extracts. There was a moderate correlation between the ORAC and ABTS•+ scavenging values and HCA content in the fruit ($0.51< R^2 <0.54$), as well as between ABTS•+ and DPPH• scavenging values and HCA content in juice extracts ($0.56< R^2 <0.62$) (Table 4 in **II**).

5.2. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs (III)

5.2.1. Sensory evaluation and *in vitro* antioxidant capacity

Preliminary analyses of the TPC values of three ingredients E, AC and R (previously described in chapter 4.2.), demonstrated that the TPC value of E was almost 5 times higher than the value of AC and 17 times higher than the value of R (Figure 1a in **III**).

The highest scores for the colour (7.8) and for the juiciness (7.5) achieved the samples with 3% of AC and with 1% of R, respectively. The samples with 1% of R got the highest score of 7.5 also for the taste and the odour. As the samples with more than 1% of E scored less than 5 for taste, they were not acceptable for panellists and were excluded from further use. The average scores of sensory attributes more than 5 were achieved by both, AC and R with concentrations 1-3% and therefore, 2% of AC and R as well as 1% of E were chosen for further experiments.

The meatballs with ingredients had remarkably higher antioxidant (DPPH•) assay values than in case of meatballs without any ingredients. The addition of 1% of E, 2% of AC, and 2% of R, increased the antioxidant potential of meatballs more than 15-, 10- and 5- times, respectively (Figure 1b **III**).

5.2.2. Proximate composition and cooking losses (III)

In current study, the plant-based ingredients did not change the moisture, ash and protein contents, due to their low concentration (1-2%). However, the fibre-rich AC and R ingredients decreased the fat content in meatballs, as well R also decreased the cooking loss more than 13%. (Table 1, in **III**). The ingredient R increased the juiciness of meatballs more than 2% compared to the control sample, while the extract E increased the cooking losses significantly.

5.2.3. Determination of physicochemical parameters (III)

The pH of meatballs with 1%-E was significantly lower compared to the control sample (P=0.0132) (Figure 3a in **III**) after 5 days of storage at 4 °C, which can be explained by chlorogenic acids present in E. The pH remained stable during the 5-days of storage in the samples with 2% of AC and 2% of R, due to some content of chlorogenic acid. However, the pH value increased in the control sample, which may indicate microbiological spoilage of meat.

The Aw values of the meatballs ranged within 0.974-0.987 (Figure 3 b in **III**). The inclusion of fibre-rich 2% of R ingredient reduced the Aw values of meatballs approximately 1.3%, however, such a small decrease doesn`t have any effect to the self-life of meatballs. The other ingredients (2% of AC and 1% E) had even lower reduction of aw, 0.7% and 0.3%, respectively.

While testing the L*a*b* values of meatballs, the rowanberry pomace based ingredients decreased the lightness (L*) (Figure 4a in **III**) and increased the redness (a*)(Figure 4b in **III**) up to 48% due to the high content of anthocyanins in the rowanberries (**II**). In addition, the ingredients 1%-E and 2% of AC decreased the yellowness (b*) of meatballs 1.87% and 0.42%, respectively, due to their high level of bioactive components. However, 2% of R increased b* value approximately 0.51%, compared to the control, during 5 days of storage (Figure 4c in **III**).

5.2.4. Untargeted chemical profiling of meatballs added with rowanberry ingredients during storage (III)

During the untargeted UHPLC-Orbitrap analysis on the meatballs with different ingredients at 3 time points 402 compounds were detected according to their individual abundance values and composite mass spectra (MSMS). The list of all compounds annotated, their corresponding mass spectra, isotopic profile, and identification-related information, is provided as (supplementary material in **III)**.

To group samples according to their intrinsic similarities in their chemical profile, the unsupervised hierarchical cluster analysis (HCA) was used; as well, the corresponding heat-map was created (Figure 4 in **III)**. The

HCA consisted of the first cluster, which hierarchically included the control samples at the day points 0, 4 and 14, and the second cluster, which showed the meatballs with ingredients 2% of AC, 1% of E, and 2% of R.

Both the heat-map (Figure 4 in **III**) as well as unsupervised PCA score plot along the first principal component (PC1) (supplementary material in **III**), demonstrated the potential effect of rowanberry pomace ingredients on modifying the meat metabolomic profile. Moreover, the unsupervised statistical findings demonstrated the impact of storage time on the chemical profile of meatball samples. For further investigation of the impact of storage time on the chemical profile of meatball samples with and without ingredients, a supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was used. The prediction models of OPLS-DA score plots (supplementary material in III) showed a clear separation trend between the different time points of storage time as well as more than acceptable goodness of fitting ($R^2Y > 0.9$) and prediction ability ($Q^2 > 0.5$) values. Subsequently, the changes in meat metabolites in the last time point (i.e., 14 days) were evaluated, to understand the potential impact of oxidative processes on meat constituents as well as the protective role of rowanberry pomace ingredients. Therefore, to get a better understanding of what kind of metabolites were in the meatball samples at the day-14 of storage, the OPLS-DA model was built. The OPLS-DA score plot (Figure 5 in **III**) demonstrated that the control sample (C14) was separated from the samples with ingredients (R14, E14, and AC14) along the orthogonal latent vector. The OPLS-DA model recorded the excellent cross-validation and goodness parameters, with R^2X (cum) = 0.719, R^2Y (cum) = 0.993, and Q^2Y (prediction ability) = 0.919. Afterwards, to select the most discriminant metabolites of the OPLS-DA model, the variable importance in projection (VIP) approach was used. This approach exposed 184 discriminant metabolites with higher than 1 (i.e., high prediction ability) VIP score. These marker compounds are listed in Table S1 (supplementary material in **III**), grouped in chemical classes given in the comprehensive database FooDB. Moreover, the Log Fold- Change (FC) variations between the meatballs with three different ingredients and the control were evaluated. Overall, the most discriminant markers were terpenoids (52 compounds), amino acids (26 compounds), fatty acid derivatives (including esters, acids, and alcohols), polyphenols (16 compounds) and some aldehydes and ketones (Table 2.)

Table 2. The chemical classes of marker compounds by the database FooDB, the number of discriminant compounds and the most discriminant compounds according to **OPLS-DA.** Variation values according to the Log Fold- Change (FC) variations between the three different treatments with the control at day-14. (unpublished data)

Class	Number of discriminant compounds (OPLS-DA)	Variation values (Fold-Change, FC)	C14 *vs* 2%-R14	C14 *vs* 1%-E14	C14 *vs* 2%-AC14	Most discriminant compound (OPLS-DA)
Aldehydes	5		0.62	1.43	0.81	3-(4-Methoxy-phenyl)-2-methyl-2-propenal (VIP score = 1.263)
		LogFC average				
		LogFC cumulative	3.13	7.16	4.01	
Amino acids, peptides, and analogues	**26**	***LogFC average***	**0.81**	**0.84**	**0.14**	L-Proline (VIP score = 1.336)
		LogFC cumulative	21.10	21.83	3.68	
Fatty acid esters	14		-0.69	0.03	-0.85	Hex-trans-3-enyl 2-methyl-butyrate (VIP score = 1.337)
		LogFC average				
		LogFC cumulative	-9.65	0.41	-11.84	
Polyphenols	**16**		**-4.83**	**-6.19**	**-5.94**	5-(10,13-Nonadeca-dienyl)-1,3-benzene-diol (VIP score = 1.332)
		LogFC average				
		LogFC cumulative	-77.25	-99.06	-95.04	
		LogFC cumulative	1.20	4.76	2.98	
Ketones	6	*LogFC average*	0.64	1.21	0.87	(E)-5-Nonen-2-one (VIP score = 1.117)
		LogFC cumulative	3.83	7.27	5.24	

Linoleic acids and derivatives	**5**	***LogFC average***	**-0.27**	**-0.42**	**-0.27**	12-Hydroxy-8,10-octadecadienoic acid (VIP score = 1.115)
		LogFC cumulative	-1.36	-2.08	-1.34	
Vitamin E and K derivatives	3	*LogFC average*	-8.54	-9.43	-9.01	gamma-tocotrienol (VIP score = 1.042)
		LogFC cumulative	-25.63	-28.28	-27.01	
Terpenoids	**52**	***LogFC average***	**-6.37**	**-6.30**	**-6.27**	2-Ethyl-1,3,3-trimethyl-2-norbornanol (VIP score = 1.333)
		LogFC cumulative	-331.25	-327.77	-326.17	
Steroid derivatives	7	*LogFC average*	-2.03	-1.99	-1.83	25-Hydroxycholesterol (VIP score = 1.075)
		LogFC cumulative	-14.24	-13.93	-12.82	

The fatty acid derivatives, such as aldehydes, and ketones accumulated in the control sample and not in the samples with ingredients in Table S1 (supplementary material in **III**). It revealed that 1% of E was the most active rowanberry ingredient against the accumulation of aldehydes and ketones, recording cumulative LogFC values of 7.16 and 7.27, respectively, compared with the control (C) at 14 days of storage time. The lipid oxidation can be discovered by the off-flavours caused by the volatile fraction of carbonyl compounds. According to (supplementary material in **III**), five discriminant aldehydic compounds with high LogFC values, such as 7-Dodecenal and 2,4-Heptadienal existed in the meatballs. In addition, the content of linoleic acid derivatives decreased in the control sample (C), thus indicating a higher lipidic peroxidation compared to that in meatballs with rowanberry pomace ingredients.

Three different pairwise comparisons revealed the accumulation of phosphatidylethanolamine (PE) derivatives, such as PE (14:1(9Z)/14:1(9Z)), PE (14:0/14:0), PE (14:0/14:1(9Z)) as well as of the steroid derivative 25-Hydroxycholesterol, which is likely correlated to lipid and cholesterol oxidative processes Table S1 (supplementary

material in **III**). Moreover, in the control sample the content of the most discriminant terpenoids (including monoterpenoids, diterpenoids, triterpenoids, tetraterpenoids, and sesquiterpenoids) and phenolic compounds (mainly flavonoids and phenolic acids) had decreased compared to the meatballs with ingredients during 14 days of storage according to their LogFC values.

The chlorogenic acid (average LogFC *vs* C = 13.98) and isoquercitrin (average LogFC *vs* C = 12.30) were the most prevalent phenolic compounds connected with the addition of rowanberry ingredients in the meatballs. In addition, some important triterpenoids, such as 3-trans-*p*-coumaroylrotundic acid (also a biomarker of blueberry) and glycyrrhetinic acid (reported for its inflammatory properties), have been identified following the addition of rowanberry ingredients.

The strong accumulation of these compounds at the end of storage time in meatballs with rowanberry pomace ingredients demonstrates that they can protect against lipid oxidation.

6. DISCUSSION

6.1. Antioxidants characterization of the fruit, juice, and pomace of sweet rowanberry (*Sorbus aucuparia* L.) cultivated in Estonia (II)

6.1.1. Total phenolic content (II)

In dark coloured hybrid cultivars, such as 'Burka', 'Likernaja', 'Granatnaja' and 'Rubinovaja,' the obtained TPC values, either in fruit, juice or pomace samples, were remarkably higher than in orange-coloured varieties (Table 2 in **II**). These findings were in agreement with the TPC values reported in the research of Kampuss *et al.* (2009), who detected the highest TPC values in the rowanberry and chokeberry hybrid (*S. aucuparia* L. × *Aronia melanocarpa*) cv 'Likernaja' (484.9 mg/100 g fw). In addition, Kampuss *et al.* (2009) detected the TPC value more than 300 mg/100 g fw in the hawthorn and rowanberry hybrid 'Granatnaja', while this value was remarkably lower in the other 6 rowanberry cultivars. Similarly, Hukkanen *et al.* (2006) analyzed nine rowan cvs and found the highest TPC values for cvs 'Rubinovaja', hybrid of rowanberry and pear (*S. aucuparia* L. × *Pyrus communis* L.) and 'Burka', hybrid of chokeberry and rowanberry (*S. aria* × *Aronia arbutifolia* = *Sorbaronia alpina*) × *S. aucuparia* L.); their respective TPC values were 1014 and 820 mg/100 g of fw of fruit.

In our experiments, especially high TPC values were detected in pomace samples. Among the other varieties, also wild rowanberry and cvs 'Solnechnaja', 'Moravica', 'Krasnaja' and 'Sahharnaja' pomace samples had relatively high TPC values compared to the other varieties. These findings prove that the pomace of hybrid cultivars, but also some other cvs could be considerable as the starting materials *in vitro* and biorefining experiments.

6.1.2. Antioxidant capacity (II)

In the current study, the antioxidant potential of rowanberry fruit, juice, and pomace samples was assessed by methods based on the capacity to scavenge ABTS•+ and DPPH• and measure the oxygen radical

absorbance capacity (ORAC). The differences in mean values of three antioxidant assays (Figure 2b in **II**) demonstrate the dissimilar reaction mechanisms influencing these assays. Although ABTS•+ reaction mechanism is still unclear and depends on individual antioxidants as well as reaction conditions, it is found to be more a mixed-mode assay reagent, which reacts by both ET (electron-) and HAT (hydrogen atom transfer) mechanisms (Apak *et al.*, 2016). This is the same with the DPPH• reaction mechanism, which depends strongly on phenol-ionizing solvents and at alkaline pH, where DPPH• is a stable radical; although is believed to act more like an H-atom acceptor, the ET mechanism cannot still be excluded (Apak *et al.*, 2016; Huang *et al.*, 2005). The ORAC assay is obviously based on the HAT reaction mechanism Prior *et al.* (2005) as ORAC measures antioxidant inhibition of peroxyl radical induced oxidations reflected by radical chain breaking antioxidant activity accompanied by H atom transfer. All three of these assays can be adapted to detect both hydrophilic and hydrophobic antioxidants.

In current study in every fruit, juice or pomace samples of hybrid cvs 'Likernaja', 'Burka', 'Rubinovaja', and 'Granatnaja' the detected antiradical scavenging values were above the average. Especially high were the antioxidant capacity values of pomace samples. Similarly to the previous studies the detected DPPH• values in cv. 'Likernaja' were the highest among the values of other cvs (Jurikova *et al.*, 2014; Kampuss *et al.*, 2009). Although the different antioxidant methods resulted with different numerical values and the highest antioxidant capacity values were detected in different rowanberry varieties, hybrid cvs still had the highest DPPH•, ABTS•+ or ORAC values. The reasons of dissimilar values of different rowanberry varieties is above-described dissimilar reaction mechanisms. The present study revealed that a number of rowans, which are less studied, such as wild rowanberry, the sweet rowanberry cvs 'Solnechnaja', 'Sahharnaja' and some others, have significantly high antioxidant capacity values. Especially, 'Solnechnaja' had the highest ORAC values of all tested pomace and juice samples and the values of the other two assays were above the average of 17 pomace samples. Comparing the numerical values obtained by different antioxidant capacity assays, it reveals that the means of ORAC and ABTS•+ values increase similarly in the direction: pomace < juice < fruit, however the DPPH• mean values increase in the direction: juice < fruit < pomace, then (Figure 2 in **II**). In this work, as expected there was detected strong correlations between all used antioxidant assays (DPPH,

ABTS and ORAC) and TPC as well as anthocyanin contents with R2 values up to 0.95 and 0.89, respectively. Similarly, in the study of M. A. Olszewska *et al.* (2012) there was detected the strong correlation for methods, DPPH and TEAC with the TPC levels. Regarding the different plant materials, such as the whole fruit, pressed juice and pomace, it reveals that the correlations between antioxidant assays and phenolic groups of cvs are slightly different. Although in the case of the fruit and juice samples, the hydroxycinnamic acids content has an additional moderate effect on radical scavenging activity with R2 values up to 0.62, no statistically significant correlations were found in case of pomace samples in this study**.**

6.1.3. Identification and quantification of individual phenolic compounds in different fractions of sweet rowanberry cultivars (II)

Sweet rowanberry cvs for present study were collected from the Polli Horticultural Research Centre of the Estonian University of Life Sciences (58°07´44.5´´N, 25°32´16.8´´E), where the region can be characterized as transition zone between maritime and continental climate with four seasons of near-equal length and 102–127 rainy days a year.

In order to identify the individual phenolic compounds in different fractions of sweet rowanberry cultivars, the extracts recovered with acidified ethanol from fruit, juice, and pomace fractions were analyzed by UHPLC-DAD-MS/MS. The results (Figure 3 and Table 3 in **II**) revealed that sweet rowanberry cvs are rich in caffeoylquinic acids, especially neochlorogenic and chlorogenic acids. In the previous study of Bobinaitė *et al.* (2020), the UPLC-ESI-MS/MS analysis was used for identifying the individual polyphenolic components in the acetone, ethanol and water extracts of sweet rowanberry pomace mixture, similarly, it was detected that neochlorogenic and chlorogenic acids were the major phenolic compounds in all extracts. Regarding the present study, in the fruit and juice samples of hybrid cvs 'Likernaja', 'Burka', 'Granatnaja' and 'Rubinovaja', the neochlorogenic acid was the most dominant phenolic compound, whereas the sweet rowanberry cvs 'Sahharnaja', 'Bussinka', 'Angri', and wild rowanberry contained larger amount of chlorogenic acid. However, Jurikova *et al.* (2014), who investigated the antioxidant properties of six hybrid cvs of sweet rowanberry cvs 'Burka', 'Granatnaja' and 'Likernaja', detected the highest content of

chlorogenic acid in cvs 'Likernaja' (100.9 mg/100 g fw) and 'Granatnaja' (90.62 mg/100 g fw). These authors focused only on specific phenolic compounds, such as gallic acid, catalposide, rutin, quercetin, chlorogenic acid and quercitrin. However, our study is in agreement with the results of Jurikova *et al.* (2014) about the high amount of chlorogenic acid in cvs 'Likernaja' and 'Granatnaja'. In addition, in the previous study of Mikulic-Petkovsek *et al.* (2017b) the biochemical profile of fruits of nine rowanberry genotypes were investigated, of which cvs 'Alaja Krupnaja', 'Burka', 'Granatnaja' and 'Bussinka' overlapped with our study, reported the highest chlorogenic acid contents in the fruits of cvs 'Krasavica' and 'Alaja Krupnaja'. These findings are not consistent with our study, which revealed that 'Bussinka' had the highest chlorogenic acid content among the selected group. Mikulic-Petkovsek *et al.* (2017b) also reported cv 'Bussinka' to be rich in neochlorogenic acid (1061 mg/kg fw). However, the current study detected the lowest content of neochlorogenic acid in cv 'Bussinka' among the selected group.

According to the correlation analysis in **II** and in the study of Zymone *et al.* (2018) it is obvious that anthocyanins have the highest contribution to the antioxidant activity. The present study revealed that cvs 'Burka' had the highest total anthocyanin content, followed by cvs 'Likernaja', 'Granatnaja' and 'Rubinovaja'. These findings are in agreement with Hukkanen *et al.* (2006) and Zymone *et al.* (2018), who also detected the highest contents of anthocyanins in the cvs 'Burka' and 'Likernaja'. In the paper of Zymone *et al.* (2018), the composition of fruit powders of 20 sweet rowanberry cvs was determined, while all the hybrid cvs were overlapping with **II.** In addition, Kylli *et al.* (2010), who investigated cvs 'Burka', 'Granatnaja' and wild rowanberry that overlap with present research, reported that anthocyanins contributed a highest amount (17.3-28.4%) of the total phenolics in the hybrid cvs 'Burka', 'Granatnaja'. In the previous study of Mikulic-Petkovsek *et al.* (2017b) the highest content of total anthocyanins was detected both in cvs 'Burka' (871 mg/kg fw) and 'Bussinka' (856 mg/kg fw), which is in agreement to the paper **II** in connection with cv 'Burka'. However, the content in cv 'Bussinka' was 10-fold lower in **II** compared to cv 'Burka'. Moreover, the qualitative analysis of 16 sweet rowanberry cultivars (cvs) and wild rowanberry in **II**, revealed that the fruit and juice of the non-hybrid rowanberry cvs had total anthocyanin contents less than 1 mg/g dw, while in hybrids the content ranged between 2.28 to 7.27 mg/g dw.

Cyanidin glucosides are a common group of anthocyanins in the rowanberries. Similarly to present study of paper **II**, Hukkanen *et al.* (2006); Kylli *et al.* (2010) and Zymone *et al.* (2018) claimed that cyanidin-3-galactoside is the predominant anthocyanin in rowanberry fruit. In addition, the paper **II** revealed that cyanidin-3-galactoside and cyanidin-3-arabinoside constitute together > 90% of the total anthocyanins in the rowanberry hybrids.

According to Mikulic-Petkovsek *et al.* (2017b), quercetin derivatives, which represent more than 95% of total flavonols, cv 'Alaja Krupnaja' had up to 29-fold higher rutin content than other analyzed genotypes. However, according to the paper **II**, cvs 'Burka', 'Likernaja' and 'Rubinovaja' had comparable rutin values with cv 'Alaja Krupnaja'. Moreover, Jurikova *et al.* (2014) detected the highest rutin content in cv 'Likernaja', however, the content in cv 'Burka' was much lower, as well cv 'Rubinovaja' and 'Alaja Krupnaja' were not determined in their study.

The discrepancies in some results, such as the different chlorogenic and neochlorogenic acid contents as well as total anthocyanin and rutin values, could be explained by different climatic conditions. The plants of Mikulic-Petkovsek *et al.* (2017b) were planted at the experimental station of Horticulture faculty in Lednice (Czech Republic) (latitude: 48° 47` 41.90`` N, longitude: 16° 47` 56.91`` E, and altitude of 176 m above sea level). According to Quitt's classification, it belongs to the T4 climatic region with extremely warm, dry, and long summers, warm springs, and autumns, short, mild, and dry to very dry winters, and a very short duration of snow cover. The rowanberries for the study of Jurikova *et al.* (2014) were harvested from an experimental gene-fund orchard of Mendel University in Brno, the Czech Republic, where the average annual temperature is 9 ºC and a fifty-year average sum of precipitation is 553 mm. While in the study of Kylli *et al.*, (2010), the cvs were grown in more Nordic conditions, Koijärvi, Finland (Latitude: 61°05`15`` N, longitude: 23° 39`33``E). Interestingly, Hukkanen *et al.* (2006) obtained the sweet rowanberry cvs as grafts from the Polli Horticultural Research Centre of the Estonian University of Life Sciences, however the micropropagated plantlets were planted in the Research Garden of the University of Kuopio, Finland, where the climate is continental, with freezing winters and mild summers.

Considering the high contents of phenolic compounds, as well as the significant free radical scavenging potential, the pomace mixture of two cvs 'Likernaja' and 'Solnechnaja' as well as wild rowanberry was chosen for further valorization and application as ingredients possessing antioxidant capacity for inhibiting the lipid oxidation in meatballs **(III).**

6.2. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs (III)

Due to the indications regarding the harmfulness of synthetic ingredients, the tendency to replace synthetic antioxidants with natural ones, in order to inhibit lipid oxidation in food, has been increasing. Therefore, various natural preservatives have recently been tested to extend the storage time of foods (Aziz & Karboune, 2018). However, the application of rowanberry, similarly to many other plant-based ingredients to food products as preservatives may be limited due to the flavour characteristics (Dussault *et al.*, 2014). The astringent taste of rowanberry, described in review paper **I**, is a major obstacle to its consumption. Therefore, it was essential to ascertain the acceptable dose of rowanberry ingredients to achieve the sensory quality of meatballs. As mentioned above, 3 rowanberry pomace powders (cvs 'Likernaja', 'Solnechnaja' and wild rowanberry) were selected as the cvs with the considerable antioxidant capacity and phenolic content **(II).** The pomace mixture was defatted by supercritical CO_2 for removing lipophilic substances at 40 MPa pressure, 40 °C temperature and 150 min extraction time, similarly to Tamkute *et al.* (2021) to obtain the 1st ingredient (AC) for meatballs. As mentioned by Venskutonis (2020a), the polyunsaturated fatty acids in berry seeds, may accelerate the formation of oxidation products in meatballs during storage, therefore lipophilic CO_2 extract was not used in the meat tests. However, AC was further extracted with 50% ethanol using microwave-assisted extraction (MAE). The 2nd (E) and 3rd (R) ingredient for meatballs were obtained by the filtration, evaporation/lyophilisation of ethanolic extract **(III).** The meatballs were prepared according to the protocol of Kerner *et al.* (2021) and after assessment by the hedonic 9-point scale of Wichchukit & O'Mahony (2015), 2% of AC and 2% of R as well as 1% of E were chosen for further experiments. Although Paglarini *et al.* (2022) has reported the positive impact of plant-based ingredients to meat products considering the better texture, moisture holding capacity as well as the reduction of the content of animal-based

proteins and saturated fatty acids, in paper **III** these effects were just marginal due to the low contents of ingredients. However, in the current study, the ingredient R reduced the cooking loss more than 13%, which is in agreement with the results of Mena *et al.* (2020), who achieved the remarkable decrease in cooking loss with 3% sugarcane fibre addition to meatballs.

According to Tamkute *et al.* (2021), the quality characteristics, which play an important role in defining consumers` preferences, are the juiciness, colour, freshness and tenderness of meat products, are affected by pH, water activity (aw) and colour (Tamkute *et al.*, 2021). Barcenilla *et al.* (2022), who investigated the application of lactic acid bacteria for the bio-preservation of meat products, claimed that the decrease in pH can lead to an unacceptable taste in food, however, it can also provide an inhibitory effect against spoilage or pathogenic microorganisms.

In the paper **III**, the decrease in pH, which was detected in the meatballs with 1% of E after 5 days of storage at 4 °C (Figure 2 a in **III**), can be explained by the chlorogenic acids present in rowanberry extract (**II**). However, in the case of the samples with 2% of AC and 2% of R, the pH remained stable during the 5-days of storage, which is in agreement with findings of Tamkute *et al.* (2021), who found that the pH of cooked ham samples with chokeberry extract remained constant during a prolonged (36 days) storage period at 4 °C. In both, the paper **III** and the study of Tamkute *et al.* (2021), the pH of control sample increased during the test period.

According to Peiretti *et al.* (2020), the discolouration of red meat could occur due to the oxidation of the iron atoms in haemoglobin redox. In the studies with chokeberry as well as with blueberries, grapes and blackcurrants and all the rowanberry pomace-based ingredients, the ingredients increased the redness (a^*) of meatballs (**III**) (Peiretti *et al.*, 2020; Tamkutė & Vaicekauskaitė, 2021). The high increase up to 48% (Figure 3 a in **III**) likely occurred due to the high content of anthocyanins in rowanberries (**II**).

Untargeted chemical profiling of meatballs with rowanberry ingredients during storage detected 184 discriminant metabolites having a VIP score higher than 1 (i.e., high prediction ability). According to Rocchetti *et al.* (2021), the five discriminant aldehydic compounds, which

were characterized by high LogFC values, such as 7-Dodecenal and 2,4-Heptadienal, have already been detected as a marker associated with oxidative processes on meat components and their overall up-accumulation could be associated with possible protective effect of the rowanberry pomace on lipid oxidation. The addition of rowanberry ingredients associated with detection of triterpenoid compounds, such as 3-trans-*p*-coumaroylrotundic acid, which has previously been detected as a biomarker of blueberry by Das *et al.* (2022), as well as glycyrrhetinic acid, which has comprehensively been studied for its inflammatory properties (Ming *et al.*, 2013). However, the major phenolic compounds associated with rowanberry ingredients in meatballs were chlorogenic acid and isoquercitrin as their distribution in rowanberries has been reported in paper **II**, as well as their antioxidant activities against free radicals have been previously reported.

7. CONCLUSIONS

In this study, the background of the big genus *Sorbus* L. was investigated by composing a review article, which revealed the most significant aspects of the rowan genotypes. Subsequently, the antioxidant effect and polyphenolic composition of fruit, juice and pomace of 16 locally grown sweet rowanberry hybrids and cultivars and wild rowanberry were investigated, in order to find the most promising cultivars with the considerable contents of polyphenols and antioxidant capacity. The pomace part of the most promising rowanberry cultivars was biorefined and applied as a beneficial functional ingredient in food product.

Based on the established hypotheses and aims, the conclusions from the present study are provided below:

- The comprehensive review (**I**) of various aspects of rowan (*Sorbus* sp.) species grown all over the world revealed that the fruit of *S. aucuparia* L.*, S. aria (*L.) Crantz*, S. sambucifolia* (Cham. et Schltdl.) M.Roem., all parts of *S. commixta* Hedl., the leaves and inflorescences *of S. gracilis* (Siebold et Zucc.) K. Koch and *S. koehneana* C. K. Schneid., and the leaves of *S. wilfordii* Koehne and *S. pogonopetala* distinguished for their significant antioxidant effect and the polyphenolic composition.
- The antioxidants characterization of 16 fruit bearing sweet rowanberry hybrids and cultivars and wild rowanberry cultivated in Estonia, revealed that the most promising cultivars with the considerable contents of polyphenols and antioxidant capacity were all the used rowanberry hybrid cvs, 'Likernaja', 'Burka', 'Granatnaja' and 'Rubinovaja', and also some seedlings of *S. aucuparia* L.*,* cv. 'Solnechnaja' and 'Sahharnaja', and wild rowanberry (**II**).
- The hypothesis that the significant part of phytochemicals with high antioxidant potential and polyphenolic content remains in the rowanberry pomace fraction, was completely confirmed (**II**), and this fraction can be a potential source of functional ingredients for the biorefining process to increase the utilization of sweet rowanberry cultivars.
- The natural ingredients possessing antioxidant capacity obtained by rowanberry pomace valorisation were defatted pomace (AC),

extraction residue (R) and lyophilized ethanolic extract (E) (**III**). The most effective rowanberry ingredient was 1% of E. During 14 days of storage test, the ingredients inhibited the development of unpleasant flavours caused by carbonyl compounds, while the concentration of linoleic acid derivatives only decreased in the control sample. These findings prove the hypothesis that the rowanberry pomace-based ingredients can be beneficial functional ingredients for food.

Future studies could focus on the following aspects:

- The simultaneous separation of rowanberry pomace lipophilic compounds isolated by SFE-CO_2 with a co-solvent (EtOH) in order to concentrate the valuable lipophilic components (β-carotene, tocopherols, phytosterols, and volatile components)
- Comparison of the different extraction methods, such as microwave-assisted extraction, ultrasound-assisted extraction, and pressurized liquid extraction, in order to obtain the extracts with the highest polyphenolic concentrations and antioxidant potential, in order to inhibit the oxidation in food.
- Optimization of the fractionation conditions, such as co-solvent concentration, time, pressure and temperature for every lipophilic component, such as β-carotene, selected phytosterols, tocopherols or volatile compounds.
- Exploration of various applications for extracts or powders obtained from rowanberries.
- Comparison of the effect of rowanberry pomace-based ingredients to the storage time of the meatballs with some more frequently used plant-based food ingredients, such as garlic, rosemary, oregano, onion, pepper.

8. REFERENCES

Acree, T., & Arnold, H. (2004, July 28). *Flavornet*. http://www.flavornet.org

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

Ahmadkelayeh, S., & Hawboldt, K. (2020). Extraction of lipids and astaxanthin from crustacean by-products: A review on supercritical CO_2 extraction. In *Trends in Food Science and Technology* (Vol. 103, pp. 94–108). Elsevier Ltd. Doi:10.1016/j.tifs.2020.07.016

Aladedunye, F., Niehaus, K., Bednarz, H., Thiyam-Hollander, U., Fehling, E., & Matthäus, B. (2015). Enzymatic lipophilization of phenolic extract from rowanberry (Sorbus aucuparia) and evaluation of antioxidative activity in edible oil. *LWT - Food Science and Technology*, *60*(1), 56–62. Doi:10.1016/j.lwt.2014.08.008

Aldasoro, J. J., Aedo, C., & Garmendia, F. (2004). Revision of Sorbus subgenera Aria and Torminaria (Rosaceae-Maloideae). *Systematic Botany Monographs*, *69*, 1–148.

Aldasoro, J. J., Aedo, C., & Muñoz, F. (1998). The Genus Sorbus (Maloideae, Rosaceae) in Europe and in North Africa: Morphological Analysis and Systematics. *American Society of Plant Taxonomists*, *23*(2), 189–212. http://www.jstor.org/page/info/about/policies/terms.jsp

Ali, E., Hussain, S., Hussain, N., Kakar, K. U., Shah, J. M., Zaidi, S. H. R., Jan, M., Zhang, K., Khan, M. A., & Imtiaz, M. (2022). Tocopherol as plant protector: an overview of Tocopherol biosynthesis enzymes and their role as antioxidant and signaling molecules. In *Acta Physiologiae Plantarum* (Vol. 44, Issue 2). Springer Science and Business Media Deutschland GmbH. Doi:10.1007/s11738-021-03350-x

Andrade, T. A., Hamerski, F., López Fetzer, D. E., Roda-Serrat, M. C., Corazza, M. L., Norddahl, B., & Errico, M. (2021). Ultrasound-assisted pressurized liquid extraction of anthocyanins from Aronia melanocarpa pomace. *Separation and Purification Technology*, *276*. Doi:10.1016/j.seppur.2021.119290

Apak, R., Özyürek, M., Güçlü, K., & Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. 2. Hydrogen atom transfer (HAT)-

based, mixed-mode (electron transfer (ET)/HAT), and lipid peroxidation assays. In *Journal of Agricultural and Food Chemistry* (Vol. 64, Issue 5, pp. 1028–1045). American Chemical Society. Doi:10.1021/acs.jafc.5b04743

Aslantas, R., Pirlak, L., & Güleryüz, M. (2007). The nutritional value of wild fruits from the North eastern Anatolia Region of Turkey. *Asian Journal of Chemistry*, *19*(4), 3072–3078.

Ayupbek, A., Hu, K.-L., & Aisa, H. A. (2012). Chemical constituents from the leaves of Sorbus tianschanica. *Chemistry of Natural Compounds*, *48*(1), 133–134.

Aziz, M., & Karboune, S. (2018). Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition*, *58*(3), 486–511. Doi:10.1080/10408398.2016.1194256

Bae, H., Yun, S. K., Jun, J. H., Yoon, I. K., Nam, E. Y., & Kwon, J. H. (2014). Assessment of organic acid and sugar composition in apricot, plumcot, plum, and peach during fruit development. *Journal of Applied Botany and Food Quality*, *87*, 24–29. Doi:10.5073/JABFQ.2014.087.004

Bae, J., Sim, G., Kim, J., Pyo, H., Yun, J., & Lee, B. (2007). Antioxidative Activity of the Hydrolytic Enzyme Treated Sorbus commixta Hedl. and its Inhibitory Effect on Matrix Metallopro-teinase-1 in UV Irradiated Human Dermal Fibroblasts. *Arch Pharm Res*, *30*(9), 1116–1123. http://apr.psk.or.kr

Bailie, A., Renaut, S., Ubalijoro, E., Guerrero-Analco, J. A., Saleem, .A., Haddad, P., Arnason, J. T., Johns, T., & Cuerrier, A. (2016). Phytogeographic and genetic variation in Sorbus, a traditional antidiabetic medicine-adaptation in action in both a plant and a discipline. *PeerJ*, *2*(11), 1–22. Doi:10.7717/peerj.2645

Baldino, L., Scognamiglio, M., & Reverchon, E. (2020). Supercritical fluid technologies applied to the extraction of compounds of industrial interest from Cannabis sativa L. and to their pharmaceutical formulations: A review. In *Journal of Supercritical Fluids* (Vol. 165). Elsevier B.V. Doi:10.1016/j.supflu.2020.104960

Barcenilla, C., Ducic, M., López, M., Prieto, M., & Álvarez-Ordóñez, A. (2022). Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. *Meat Science*, *183*. Doi:10.1016/J.MEATSCI.2021.108661

Basegmez, H. I. O., Povilaitis, D., Kitrytė, V., Kraujalienė, V., Šulniūtė, V., Alasalvar, C., & Venskutonis, P. R. (2017). Biorefining of blackcurrant pomace into high value functional ingredients using supercritical CO_2, pressurized liquid and enzyme assisted extractions. *Journal of Supercritical Fluids*, *124*, 10–19. Doi:10.1016/j.supflu.2017.01.003

Berna, E., Kampuse, S., & Straumite, E. (2012). The suitability of different rowanberry cultivars for production of fruit marmalade. In Annual 18th International Scientific Conference "Research for Rural Development"; Jelgava, Latvia, 16-18 May 2012; Volume 1, Treija, S., Skuja, I. Eds.; Latvia University of Agriculture, Jelgava, Latvia. *Food Sciences*, 109–116.

Bernini, R., Carastro, I., Palmini, G., Tanini, A., Zonefrati, R., Pinelli, P., Brandi, M. L., & Romani, A. (2017). Lipophilization of hydroxytyrosol-enriched fractions from Olea europaea L. byproducts and evaluation of the in vitro effects on a model of colorectal cancer cells. *Journal of Agricultural and Food Chemistry*, *65*(31), 6506–6512. Doi:10.1021/acs.jafc.6b05457

Bhatt, L. R., Bae, M. S., Kim, B. M., Oh, G. S., & Chai, K. Y. (2009). A chalcone glycoside from the fruits of Sorbus commixta Hedl. *Molecules*, *14*(12), 5323–5327. Doi:10.3390/molecules14125323

Boath, A. S., Stewart, D., & McDougall, G. J. (2012). Berry components inhibit α-glucosidase in vitro: Synergies between acarbose and polyphenols from black currant and rowanberry. *Food Chemistry*, *135*(3), 929–936. Doi:10.1016/j.foodchem.2012.06.065

Bobinaitė, R., Grootaert, C., Van Camp, J., Šarkinas, A., Liaudanskas, M., Žvikas, V., Viškelis, P., & Rimantas Venskutonis, P. (2020). Chemical composition, antioxidant, antimicrobial and antiproliferative activities of the extracts isolated from the pomace of rowanberry (Sorbus aucuparia L.). *Food Research International*, *136*, 109310. Doi:10.1016/j.foodres.2020.109310

Bobinaite, R., Kraujalis, P., Tamkute, L., Urbonavičienė, D., Viškelis, P., & Venskutonis, P. R. (2020). Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents. *Journal of Industrial and Engineering Chemistry*, *85*, 152–160. Doi:10.1016/j.jiec.2020.01.036

Bobinaitė R, Grootaert C, Van Camp J, Šarkinas A., Liaudanskas M., Žvikas V., Viškelis P., & Rimantas V. P. (2020). Chemical composition, antioxidant, antimicrobial and antiproliferative activities of the extracts

isolated from the pomace of rowanberry (Sorbus aucuparia L.). *Food Research International*, 109310. Doi:10.1016/j.foodres.2020.109310

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*, 25–30.

Broholm, S. L., Gramsbergen, S. M., Nyberg, N. T., Jäger, A. K., & Staerk, D. (2019). Potential of Sorbus berry extracts for management of type 2 diabetes: Metabolomics investigation of 1H NMR spectra, α-amylase and α-glucosidase inhibitory activities, and in vivo anti-hyperglycaemic activity of S. norvegica. *Journal of Ethnopharmacology*, *242*. Doi:10.1016/j.jep.2019.112061

Chaimaa, M. (2021). *Chemical and biological analysis of Tramazeira*. Instituto Politecnico de Braganca (Portugal) ProQuest Dissertations Publishing. 30208608.

Cheon, S. M., Jang, I., Lee, M. H., Kim, D. K., Jeon, H., & Cha, D. S. (2017). Sorbus alnifolia protects dopaminergic neurodegeneration in Caenorhabditis elegans. *Pharmaceutical Biology*, *55*(1), 481–486. Doi:10 .1080/13880209.2016.1251468

Das, P. R., Darwish, A. G., Ismail, A., Haikal, A. M., Gajjar, P., Balasubramani, S. P., Sheikh, M. B., Tsolova, V., Soliman, K. F. A., Sherif, S. M., & El-Sharkawy, I. (2022). Diversity in blueberry genotypes and developmental stages enables discrepancy in the bioactive compounds, metabolites, and cytotoxicity. *Food Chemistry*, *374*. Doi:10.1016/j.foodchem.2021.131632

Davis, E. J., Andreani, E. S., & Karboune, S. (2021). Production of Extracts Composed of Pectic Oligo/Polysaccharides and Polyphenolic Compounds from Cranberry Pomace by Microwave-Assisted Extraction Process. *Food and Bioprocess Technology* , *14*, 634–649. Doi:10.1007/s11947-021-02593-3/Published

De Ancos, B., Colina-Coca, C., González-Peña, D., & Sánchez-Moreno, C. (2015). Bioactive compounds from vegetable and fruit by-products. In *Biotechnology of Bioactive Compounds: Sources and applications* (pp. 1–34). John Wiley & Sons: Hoboken, NJ, USA. Doi:10.1002/9781118733103.ch1

Denev, P., Kratchanova, M., Ciz, M., & Lojek, A. (2014). Antioxidant, antimicrobial and neutrophil-modulating activities of herb extracts. *Acta Biochimica Polonica*, *61*(2), 359–367.

Dolezal, M., Velisek, J., & Famfulikova, P. (2003). Aroma of less-known wild fruits. *Flavour Res. Dawn Twenty-First Century, Proc. Weurman Flavor Res. Symp., 10th*, 576–579.

Doležal, M., Velíšek, J., & Famfulikova, P. (2003). Aroma of less-known wild fruits. *Flavour Research at the Dawn of the Twenty-First Century-Proceedings of the 10th Weurman Flavour Research Symposium, Beaune, France, 25-28 June, 2002. Editions Tec & Doc*, 576–579.

Doležal, M., Velíšek, J., & Famfulíková, P. (2001). Chemical composition of less-known wild fruits. *Biologically-Active Phytochemicals in Food: Analysis, Metabolism, Bioavailability and Function. Proceedings of the EUROFOODCHEM XI Meeting, Norwich, UK, 26-28 September 2001. Royal Society of Chemistry*, 241–244.

Dussault, D., Vu, K. D., & Lacroix, M. (2014). In vitro evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. *Meat Science*, *96*(1), 514–520. Doi:10.1016/j.meatsci.2013.08.015

Ekezie, F. G. C., Sun, D. W., & Cheng, J. H. (2017). Acceleration of microwave-assisted extraction processes of food components by integrating technologies and applying emerging solvents: A review of latest developments. In *Trends in Food Science and Technology* (Vol. 67, pp. 160–172). Elsevier Ltd. Doi:10.1016/j.tifs.2017.06.006

Ekin, H. N., Gokbulut, A., Aydin, Z. U., Donmez, A. A., & Orhan, I. E. (2016). Insight into anticholinesterase and antioxidant potential of thirty-four Rosaceae samples and phenolic characterization of the active extracts by HPLC. *Industrial Crops and Products*, *91*, 104–113. Doi:10.1016/j.indcrop.2016.06.029

Erbil, N. (2022). Potential Antibacterial Effect and L-Ascorbic Acid and Phenolic Content Profiles of Wild Rowanberry (Sorbus aucuparia L.). *Erwerbs-Obstbau*. Doi:10.1007/s10341-022-00736-0

Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *The Journal of Biological Chemistry*, *2*, 627–650.

Fomenko, S. E., Kushnerova, N. F., Sprygin, V. G., Drugova, E. S., & Momot, T. V. (2016). Chemical composition and biological action of rowanberry extract. *Russian Journal of Bioorganic Chemistry*, *42*(7), 764–769. Doi:10.1134/S1068162016070074

Gil-Izquierdo, A., & Mellenthin, A. (2001). Identification and quantitation of flavonols in rowanberry (Sorbus aucuparia L.) juice. *European Food Research and Technology*, *213*(1), 12–17. Doi:10.1007/s002170100328

Goodman, J., Adams, T., Cohen, S., Doull, J., Feron, V., Marnett, L., & . (2005). The FEMA GRAS assessment of benzyl derivatives used as flavor ingredients. In *Food and Chemical Toxicology* (Vol. 43, Issue 8, pp. 1207–1240). Doi:10.1016/j.fct.2004.11.014

Grunovaite, L., Pukalskiene, M., Pukalskas, A., & Venskutonis, P. R. (2016). Fractionation of black chokeberry pomace into functional ingredients using high pressure extraction methods and evaluation of their antioxidant capacity and chemical composition. *Journal of Functional Foods*, *24*, 85–96. Doi:10.1016/j.jff.2016.03.018

Gu, H., Chen, F., Zhang, Q., & Zang, J. (2016). Application of ionic liquids in vacuum microwave-assisted extraction followed by macroporous resin isolation of three flavonoids rutin, hyperoside and hesperidin from Sorbus tianschanica leaves. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1014*, 45–55. Doi:10.1016/j.jchromb.2016.01.045

Han, X., Shen, T., & Lou, H. (2007). Dietary Polyphenols and Their Biological Significance. *Int. J. Mol. Sci*, *8*, 950–988. http://www.mdpi.org/ijms

Herrero, M., Mendiola, J. A., Cifuentes, A., & Ibáñez, E. (2010). Supercritical fluid extraction: Recent advances and applications. In *Journal of Chromatography A* (Vol. 1217, Issue 16, pp. 2495–2511). Doi:10.1016/j.chroma.2009.12.019

Hong, S. Y., Lansky, E., Kang, S. S., & Yang, M. (2021). A review of pears (Pyrus spp.), ancient functional food for modern times. In *BMC Complementary Medicine and Therapies* (Vol. 21, Issue 1). BioMed Central Ltd. Doi:10.1186/s12906-021-03392-1

Houlberg, S., Westh, B. C., & Poll, L. (2000). The aroma composition of ethanol extracts of rowanberries (Sorbus aucuparia). *Frontiers of Flavour Science*, 56–57.

Huang, D., Boxin, O. U., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. In *Journal of Agricultural and Food Chemistry* (Vol. 53, Issue 6, pp. 1841–1856). Doi:10.1021/jf030723c

Hukkanen, A. T., Pölönen, S. S., Kärenlampi, S. O., & Kokko, H. I. (2006). Antioxidant capacity and phenolic content of sweet rowanberries.

Journal of Agricultural and Food Chemistry, *54*(1), 112–119. Doi:10.1021/jf051697g

Isaikina, N. V., Kalinkina, G. I., Razina, T. G., Zueva, E. P., Rybalkina, O. Y., Ulirich, A. V., Fedorova, E. P., & Shilova, A. B. (2018). Sorbus aucuparia L. fruit Is a source of the drug for increasing the efficiency of tumor chemotherapy. *Russian Journal of Bioorganic Chemistry*, *44*(7), 899–905. Doi:10.1134/S1068162018070038

Ivakhnov, A. D., Sadkova, K. S., Sobashnikova, A. S., & Skrebets, T. E. (2019). Optimization of Oil Extraction from Rowanberry Waste in Alcoholic Beverage Production. *Russian Journal of Physical Chemistry B*, *13*(7), 1135–1138. Doi:10.1134/S1990793119070091

Jacobo-Velázquez, D. A., & Cisneros-Zevallos, L. (2009). Correlations of antioxidant activity against phenolic content revisited: A new approach in data analysis for food and medicinal plants. *Journal of Food Science*, *74*(9). Doi:10.1111/j.1750-3841.2009.01352.x

Jiang, Y., Zhu, Y., Li, F., Du, J., Huang, Q., Sun-Waterhouse, D., & Li, D. (2020). Antioxidative pectin from hawthorn wine pomace stabilizes and protects Pickering emulsions via forming zein-pectin gel-like shell structure. *International Journal of Biological Macromolecules*, *151*, 193–203. Doi:10.1016/j.ijbiomac.2020.02.164

Jurikova, T., Sochor, J., Mlcek, J., Balla, S., Klejdus, B., Baron, M., Ercisli, S., & Ozturk Yilmaz, S. (2014). Polyphenolic profile of interspecific crosses of rowan (sorbus aucuparia L.). *Ital. J. Food Sci*, *26*, 317–324.

Kähkönen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry*, *49*(8), 4076–4082. Doi:10.1021/jf010152t

Kalle, R., & Sõukand, R. (2012). Historical ethnobotanical review of wild edible plants of Estonia (1770s-1960s). In *Acta Societatis Botanicorum Poloniae* (Vol. 81, Issue 4, pp. 271–281). Polish Botanical Society. Doi:10.5586/asbp.2012.033

Kampuss, K., Kampuse, S., BerHa, E., Krūma, Z., Krasnova, I., & Drudze, I. (2009). Biochemical composition and antiradical activity of rowanberry (SORBUS L.) cultivars and hybrids with different Rosaceae L. cultivars. *European Journal of Horticultural Science*, *59*(4), 195–201. www.blueberry.org/blueberries.htm

Kashyap, P., Riar, C. S., & Jindal, N. (2022). Polyphenol bio-accessibility and antioxidant activity of in vitrodigested ultrasound-assisted

Meghalayan cherry (Prunus nepalensis)pomace extract. *Biomass Conversion and Biorefinery*. Doi:10.1007/s13399-021-02150-0

Kenneth R. Robertson, James B. Phipps, J. R. R. and P. G. S. (1991). A Synopsis of Genera in Maloideae. In *Source: Systematic Botany* (Vol. 16, Issue 2).

Kerner, K., Jõudu, I., Tänavots, A., & Venskutonis, P. R. (2021). Application of raw and defatted by supercritical CO_2 hemp seed press-cake and sweet grass antioxidant extract in pork burger patties. *Foods*, *10*(8). Doi:10.3390/foods10081904

Khan, S., Fatima, I., Kazmi, M. H., Malik, A., Afza, N., Iqbal, L., & Latif, M. (2015). Cashmins A and B, Potent Antioxidant Coumarins from Sorbus cashmiriana. *Chemistry of Natural Compounds*, *51*(4), 626–629. Doi:10.1007/s10600-015-1370-0

Kim, M. B., Park, J. S., & Lim, S. Bin. (2010). Antioxidant activity and cell toxicity of pressurised liquid extracts from 20 selected plant species in Jeju, Korea. *Food Chemistry*, *122*(3), 546–552. Doi:10.1016/j.foodchem.2010.03.007

Kitryte, V., Laurinavičiene, A., Syrpas, M., Pukalskas, A., & Venskutonis, P. R. (2020). Modeling and optimization of supercritical carbon dioxide extraction for isolation of valuable lipophilic constituents from elderberry (Sambucus nigra L.) pomace. *Journal of CO_2 Utilization*, *35*, 225–235. Doi:10.1016/j.jcou.2019.09.020

Klavins, L., & Klavins, M. (2020). Cuticular wax composition of wild and cultivated northern berries. *Foods*, *9*(5). Doi:10.3390/foods9050587

Klavins, L., Kviesis, J., Steinberga, I., Klavina, L., & Klavins, M. (2016). Gas chromatography-mass spectrometry study of lipids in northern berries. *Agronomy Research*, *14*(S2), 1328–1346.

Kraujalis, P., & Venskutonis, P. R. (2013). Supercritical carbon dioxide extraction of squalene and tocopherols from amaranth and assessment of extracts antioxidant activity. *Journal of Supercritical Fluids*, *80*, 78–85. Doi:10.1016/j.supflu.2013.04.005

Kryževičiute, N., Kraujalis, P., & Venskutonis, P. R. (2016). Optimization of high pressure extraction processes for the separation of raspberry pomace into lipophilic and hydrophilic fractions. *Journal of Supercritical Fluids*, *108*, 61–68. Doi:10.1016/j.supflu.2015.10.025

Kylli, P., Nohynek, L., Puupponen-Pimiä, R., Westerlund-Wikström, B., McDougall, G., Stewart, D., & Heinonen, M. (2010). Rowanberry phenolics: compositional analysis and bioactivities. *Journal of Agricultural and Food Chemistry*, *58*(22), 11985–11992. Doi:10.1021/jf102739v

Lee. (2015). Sorbitol, Rubus fruit, and misconception. In *Food Chemistry* (Vol. 166, pp. 616–622). Elsevier Ltd. Doi:10.1016/j.foodchem.2014.06.073

Lee, T. K., Roh, H. S., Yu, J. S., Kwon, D. J., Kim, S. Y., Baek, K. H., & Kim, K. H. (2017). A novel cytotoxic activity of the fruit of Sorbus commixta against human lung cancer cells and isolation of the major constituents. *Journal of Functional Foods*, *30*, 1–7. Doi:10.1016/j.jff.2017.01.003

Letellier, M., & Budzinski, H. (1999). Microwave assisted extraction of organic compounds. *Analusis*, *27*(3), 259–271. Doi:10.1051/analusis:1999116

Li, H., Matsuura, M., Li, W., Li, Q., Zhang, Q., & Koike, K. (2012). Chemical constituents from the fruits of Sorbus pohuashanensis. *Biochemical Systematics and Ecology*, *43*, 166–168. Doi:10.1016/j.bse.2012.03.011

Luuk, O. (2021). 2022. aasta puu on pihlakas. *Estonian Nature*.

Majić, B., Šola, I., Likić, S., Cindrić I. J., & Rusak, G. (2015). Characterisation of Sorbus domestica (1). *Food Technol. Biotechnol.*, *53*(4), 463–471.

Mccune, L., Johns, T., & . (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. *Journal of Ethnopharmacology* , *82*, 197–205. www.elsevier.com/locate/jethpharm

Mena, B., Fang, Z., Ashman, H., Hutchings, S., Ha, M., Shand, P. J., & Warner, R. D. (2020). Influence of cooking method, fat content and food additives on physicochemical and nutritional properties of beef meatballs fortified with sugarcane fibre. *International Journal of Food Science and Technology*, *55*(6), 2381–2390. Doi:10.1111/ijfs.14482

Mikulic-Petkovsek, M., Krska, B., Kiprovski, B., & Veberic, R. (2017a). Bioactive components and antioxidant capacity of fruits from nine Sorbus genotypes. *Journal of Food Science*, *82*(3), 647–658. Doi:10.1111/1750-3841.13643

Mikulic-Petkovsek, M., Krska, B., Kiprovski, B., & Veberic, R. (2017b). Bioactive Components and Antioxidant Capacity of Fruits from Nine Sorbus Genotypes. *Journal of Food Science*, *82*(3), 647–658. Doi:10.1111/1750-3841.13643

Miletic, R., & Paunovic, S. M. (2012). Research into service tree (Sorbus domestica L.) population in eastern Serbia. *Genetika*, *44*(3), 483–490.

Minato Nakazawa, M. (2022). *Package "fmsb" Title Functions for Medical Statistics Book with some Demographic Data Depends R (>= 2.2.0)* (pp. 1–66). https://minato.sip21c.org/msb/

Ming, J., Yoke, C., & Yin, A. (2013). Therapeutic Effects of Glycyrrhizic Acid. *Natural Product Communications*, *8*(3), 415–418.

Mokrzycki, W. S., & Tatol, M. (2012). Color difference Delta E-A survey Colour difference ΔE-A survey. *Machine Graphics and Vision*, 1–28. https://www.researchgate.net/publication/236023905

Moon, S. C., Choi, H. J., Chung, T. W., Lee, J. H., Lee, S. O., Jung, M. H., Kim, B. J., Choi, J. Y., & Ha, K. T. (2018). Sorbus commixta water extract induces apoptotic cell death via a ROS-dependent pathway. *Oncology Letters*, *16*(4), 4193–4200. Doi:10.3892/ol.2018.9217

Moore, G., Goldman, D., & Garland, M. (2019). *http://plants.usda.gov*. Http://Plants.Usda.Gov.

Mrkonjić, Z. O., Nađpal, J. D., Beara, I. N., Sabo, V. S. A., Četojević-Simin, D. D., Mimica-Dukić, N. M., & Lesjak, M. M. (2017). Phenolic profiling and bioactivities of fresh fruits and jam of Sorbus species. *Journal of the Serbian Chemical Society*, *82*(6), 651–664. Doi:10.2298/JSC170202049M

Mu, H., & Høy, C. E. (2004). The digestion of dietary triacylglycerols. In *Progress in Lipid Research* (Vol. 43, Issue 2, pp. 105–133). Elsevier Ltd. Doi:10.1016/S0163-7827(03)00050-X

Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. In *Analytica Chimica Acta* (Vol. 703, Issue 1, pp. 8–18). Doi:10.1016/j.aca.2011.07.018

Nattagh-Eshtivani, E., Barghchi, H., Pahlavani, N., Barati, M., Amiri, Y., Fadel, A., Khosravi, M., Talebi, S., Arzhang, P., Ziaei, R., & Ghavami, A. (2022). Biological and pharmacological effects and nutritional impact of phytosterols: A comprehensive review. In *Phytotherapy*

Research (Vol. 36, Issue 1, pp. 299–322). John Wiley and Sons Ltd. Doi:10.1002/ptr.7312

Olszewska, M. (2008). Separation of quercetin, sexangularetin, kaempferol and isorhamnetin for simultaneous HPLC determination of flavonoid aglycones in inflorescences, leaves and fruits of three Sorbus species. *Journal of Pharmaceutical and Biomedical Analysis*, *48*(3), 629–635. Doi:10.1016/j.jpba.2008.06.004

Olszewska, M. A., Nowak, S., Michel, P., Banaszczak, P., & Kicel, A. (2010). Assessment of the content of phenolics and antioxidant action of inflorescences and leaves of selected species from the genus sorbus sensu stricto. *Molecules*, *15*(12), 8769–8783. Doi:10.3390/molecules15128769

Olszewska, M. A., Presler, A., & Michel, P. (2012). Profiling of phenolic compounds and antioxidant activity of dry extracts from the selected Sorbus species. *Molecules*, *17*(3), 3093–3113. Doi:10.3390/molecules17033093

Olszewska, M., Presler, A., & Michel, P. (2012). Profiling of phenolic compounds and antioxidant activity of dry extracts from the selected Sorbus species. *Molecules*, *17*(3), 3093–3113. Doi:10.3390/molecules17033093

Paglarini, C. de S., Vidal, V. A. S., Martini, S., Cunha, R. L., & Pollonio, M. A. R. (2022). Protein-based hydrogelled emulsions and their application as fat replacers in meat products: A review. In *Critical Reviews in Food Science and Nutrition* (Vol. 62, Issue 3, pp. 640–655). Taylor and Francis Ltd. Doi:10.1080/10408398.2020.1825322

Pasko, P. (2012). South Siberian fruits: Their selected chemical constituents, biological activity, and traditional use in folk medicine and daily nutrition. *Journal of Medicinal Plants Research*, *6*(31), 4698–4706. Doi:10.5897/jmpr12.874

Pasquel Reátegui, J. L., Machado, A. P. D. F., Barbero, G. F., Rezende, C. A., & Martínez, J. (2014). Extraction of antioxidant compounds from blackberry (Rubus sp.) bagasse using supercritical CO_2 assisted by ultrasound. *Journal of Supercritical Fluids*, *94*, 223–233. Doi:10.1016/j.supflu.2014.07.019

Pateiro, M., Vargas, F. C., Chincha, A. A. I. A., Sant'Ana, A. S., Strozzi, I., Rocchetti, G., Barba, F. J., Domínguez, R., Lucini, L., do Amaral Sobral, P. J., & Lorenzo, J. M. (2018). Guarana seed extracts as a useful

strategy to extend the shelf life of pork patties: UHPLC-ESI/QTOF phenolic profile and impact on microbial inactivation, lipid and protein oxidation and antioxidant capacity. *Food Research International*, *114*, 55–63. Doi:10.1016/j.foodres.2018.07.047

Peiretti, P. G., Gai, F., Zorzi, M., Aigotti, R., & Medana, C. (2020). The effect of blueberry pomace on the oxidative stability and cooking properties of pork patties during chilled storage. *Journal of Food Processing and Preservation*, *44*(7). Doi:10.1111/jfpp.14520

Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J., & Lampi, A. M. (2000). Plant sterols: Biosynthesis, biological function and their importance to human nutrition. In *Journal of the Science of Food and Agriculture* (Vol. 80, Issue 7, pp. 939–966). Doi:10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C

Pingret D, Chemat F, & Fabiano-Tixier A-S. (2017). Ultrasound-assisted Extraction. In *Natural Product Extraction Principles and Applications* (pp. P001–P004). Doi:10.1039/9781849737579-fp001

Polyphenols Market Growth & Trends. (2022). https://www.grandviewresearch.com/industry-analysis/polyphenols-market-

Poyrazoğlu, E. S. (2004). Changes in ascorbic acid and sugar content of rowanberries during ripening. *Journal of Food Quality*, *27*(5), 366–370. Doi:10.1111/j.1745-4557.2004.00658.x

Presler, A., Olszewska, M., & Michel, P. (2012). Profiling of phenolic compounds and antioxidant activity of dry extracts from the selected Sorbus species. *Molecules*, *17*(3), 3093–3113. Doi:10.3390/molecules17033093

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Hampsch-Woodill, M., Huang, D., Ou, B., & Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, *51*(11), 3273–3279. Doi:10.1021/jf0262256

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. In *Journal of Agricultural and Food Chemistry* (Vol. 53, Issue 10, pp. 4290–4302). Doi:10.1021/jf0502698

Pukalskienė, M., Pukalskas, A., Dienaitė, L., Revinytė, S., Pereira, C. V., Matias, A. A., & Venskutonis, P. R. (2021). Recovery of bioactive

compounds from strawberry (Fragaria × ananassa) pomace by conventional and pressurized liquid extraction and assessment their bioactivity in human cell cultures. *Foods*, *10*(8). Doi:10.3390/foods10081780

Raspé, O., Findlay, C., & Jacquemart, A. L. (2000). Sorbus aucuparia L. *Journal of Ecology*, *88*(5), 910–930. Doi:10.1046/j.1365-2745.2000.00502.x

Räty, M., Caudullo, G., & De Rigo, D. (2016). Sorbus aucuparia Sorbus aucuparia in Europe: distribution, habitat, usage and threats. *European Atlas of Forest Tree Species*, 176–177. http://www.cabi.org

Raudonis, R., Raudone, L., Gaivelyte, K., Viškelis, P., & Janulis, V. (2014). Phenolic and antioxidant profiles of rowan (Sorbus L.) fruits. *Natural Product Research*, *28*(16), 1231–1240. Doi:10.1080/14786419.2014.895727

Razina, T. G., Zueva, E. P., Ulrich, A. V., Rybalkina, O. Y., Chaikovskii, A. V., Isaikina, N. V., Kalinkina, G. I., Zhdanov, V. V., & Zyuz'Kov, G. N. (2016). Antitumor effects of sorbus aucuparia L. Extract highly saturated with anthocyans and their mechanisms. *Bulletin of Experimental Biology and Medicine*, *162*(1), 93–97. Doi:10.1007/s10517-016-3554-4

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine , , 26*, 1231–1237.

Robertson, K. R., Phipps, J. B., Rohrer, J. R., & Smith, P. G. (1991a). A Synopsis of Genera in Maloideae. In *Source: Systematic Botany* (Vol. 16, Issue 2).

Robertson, K. R., Phipps, J. B., Rohrer, J. R., & Smith, P. G. (1991b). A synopsis of genera in maloideae (Rosaceae). *Systematic Botany*, *16*(2), 376–394.

Rocchetti, G., Bernardo, L., Pateiro, M., Barba, F. J., Munekata, P. E. S., Trevisan, M., Lorenzo, J. M., & Lucini, L. (2020). Impact of a pitanga leaf extract to prevent lipid oxidation processes during shelf life of packaged pork burgers: An untargeted metabolomic approach. *Foods*, *9*(11). Doi:10.3390/foods9111668

Rocchetti, G., Michelini, S., Pizzamiglio, V., Masoero, F., & Lucini, L. (2021). A combined metabolomics and peptidomics approach to

discriminate anomalous rind inclusion levels in Parmigiano Reggiano PDO grated hard cheese from different ripening stages. *Food Research International*, *149*. Doi:10.1016/j.foodres.2021.110654

Routray, W., & Orsat, V. (2012). Microwave-Assisted Extraction of Flavonoids: A Review. In *Food and Bioprocess Technology* (Vol. 5, Issue 2, pp. 409–424). Doi:10.1007/s11947-011-0573-z

Rutkowska, M., Olszewska, M. A., Kolodziejczyk-Czepas, J., Nowak, P., & Owczarek, A. (2019). Sorbus domestica Leaf Extracts and Their Activity Markers: Antioxidant potential and synergy effects in scavenging assays of multiple oxidants. *Molecules*, *24*(12). Doi:10.3390/molecules24122289

Sadeer, N. B., Montesano, D., Albrizio, S., Zengin, G., & Mahomoodally, M. F. (2020). The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. In *Antioxidants* (Vol. 9, Issue 8, pp. 1–39). MDPI. Doi:10.3390/antiox9080709

Salek, R. M., Steinbeck, C., Viant, M. R., Goodacre, R., & Dunn, W. B. (2013). The role of reporting standards for metabolite annotation and identification in metabolomic studies. *GigaScience*, *2*(1). Doi:10.1186/2047-217X-2-13

Sarv, V., Venskutonis, P. R., Rätsep, R., Aluvee, A., Kazernavičiūtė, R., & Bhat, R. (2021). Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (Sorbus aucuparia L.) Cultivated in Estonia. *Antioxidants*, *10*(11), 1779. Doi:10.3390/antiox10111779

Šavikin, K. P., Zdunić, G. M., Krstić-Milošević, D. B., Šircelj, H. J., & Stešević, D. D. (2017). Sorbus aucuparia and Sorbus aria as a source of antioxidant phenolics, tocopherols, and pigments. *Chemistry and Biodiversity*, *14*(12), 1–11. Doi:10.1002/cbdv.201700329

Searle, S. R., Speed, F. M., & Milliken, G. A. (1980). Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. *The American Statistician*, *34*(4), 216–221. Doi:10.1080/00031305.1980.10483031

Serpen, A., Capuano, E., Fogliano, V., & Gökmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. *Journal of Agricultural and Food Chemistry*, *55*(19), 7676–7681. Doi:10.1021/jf071291z

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent . *Methods in Enzymology* , *299*, 152–178.

Slavin, M., & Yu, L. (2012). A single extraction and HPLC procedure for simultaneous analysis of phytosterols, tocopherols and lutein in soybeans. *Food Chemistry*, *135*(4), 2789–2795. Doi:10.1016/j.foodchem.2012.06.043

Sokolov, V. V., Savel'ev, N. I., & Goncharov, N. P. (2015). I.V. Michurin'S work on expansion of the plant horticulture assortment and improvement of food quality. *Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact, and Applied Sciences*, *69*(4), 190–197. Doi:10.1515/prolas-2015-0028

Sołtys, A., Galanty, A., & Podolak, I. (2020). Ethnopharmacologically important but underestimated genus Sorbus: a comprehensive review. In *Phytochemistry Reviews* (Vol. 19, Issue 2, pp. 491–526). Springer. Doi:10.1007/s11101-020-09674-9

Sukhija, P. S., & Palmquist, D. L. (1988). Rapid Method for Determination of Total Fatty Acid Content and Composition of Feedstuffs and Feces. *Journal of Agricultural and Food Chemistry,* 36, 1202–1206. https://pubs.acs.org/sharingguidelines

Tamkutė, L., Liepuoniūtė, R., Pukalskienė, M., & Venskutonis, P. R. (2020). Recovery of valuable lipophilic and polyphenolic fractions from cranberry pomace by consecutive supercritical CO_2 and pressurized liquid extraction. *Journal of Supercritical Fluids*, *159*. Doi:10.1016/j.supflu.2020.104755

Tamkutė, L., Pukalskas, A., Syrpas, M., Urbonavičienė, D., Viškelis, P., & Venskutonis, P. R. (2020). Fractionation of cranberry pomace lipids by supercritical carbon dioxide extraction and on-line separation of extracts at low temperatures. *Journal of Supercritical Fluids*, *163*. Doi:10.1016/j.supflu.2020.104884

Tamkutė, L., & Vaicekauskaitė, R. (2021). Black chokeberry (Aronia melanocarpa L.) pomace extracts inhibit food pathogenic and spoilage bacteria and increase the microbiological safety of pork products. *Journal of Food Processing and Preservation*, *45*(3). Doi:10.1111/jfpp.15220

Tamkute, L., Vaicekauskaitė, R., Melero, B., Jaime, I., Rovira, J., & Venskutonis, P. R. (2021). Effects of chokeberry extract isolated with pressurized ethanol from defatted pomace on oxidative stability, quality and sensory characteristics of pork meat products. *LWT*, *150*. Doi:10.1016/j.lwt.2021.111943

Termentzi, A., Alexiou, P., Demopoulos, V., & Kokkalou, E. (2008). The aldose reductase inhibitory capacity of Sorbus domestica fruit extracts depends on their phenolic content and may be useful for the control of diabetic complications. *Pharmazie*, *63*(9), 693–696. Doi:10.1691/ph.2008.8567

Termentzi, A., Kefalas, P., & Kokkalou, E. (2008). LC-DAD-MS (ESI+) analysis of the phenolic content of Sorbus domestica fruits in relation to their maturity stage. *Food Chemistry*, *106*(3), 1234–1245. Doi:10.1016/j.foodchem.2007.07.021

The Good Scents Company Information System. (2022, July 28). http://www.thegoodscentscompany.com/

Tian, Y., Liimatainen, J., Alanne, A. L., Lindstedt, A., Liu, P., Sinkkonen, J., Kallio, H., & Yang, B. (2017). Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chemistry*, *220*, 266–281. Doi:10.1016/j.foodchem.2016.09.145

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., Kanazawa, M., Vandergheynst, J., Fiehn, O., & Arita, M. (2015). MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nature Methods*, *12*(6), 523–526. Doi:10.1038/nmeth.3393

Tsugawa, H., Kind, T., Nakabayashi, R., Yukihira, D., Tanaka, W., Cajka, T., Saito, K., Fiehn, O., & Arita, M. (2016). Hydrogen Rearrangement Rules: Computational MS/MS Fragmentation and Structure Elucidation Using MS-FINDER Software. *Analytical Chemistry*, *88*(16), 7946–7958. Doi:10.1021/acs.analchem.6b00770

Venskutonis, P. R. (2020). Berries. In *Valorization of Fruit Processing By-products* (pp. 95–125). Elsevier. Doi:10.1016/b978-0-12-817106-6.00005-8 Doi:10.1016/b978-0-12-817106-6.00005-8

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry - A review. *Innovative Food Science and Emerging Technologies*, *9*(2), 161–169. Doi:10.1016/j.ifset.2007.04.014

Welke, J. E., Zanus, M., Lazzarotto, M., & Alcaraz Zini, C. (2014). Quantitative analysis of headspace volatile compounds using comprehensive two-dimensional gas chromatography and their contribution to the aroma of Chardonnay wine. *Food Research International*, *59*, 85–99. Doi:10.1016/j.foodres.2014.02.002

Wichchukit, S., & O'Mahony, M. (2015). The 9-point hedonic scale and hedonic ranking in food science: Some reappraisals and alternatives. In *Journal of the Science of Food and Agriculture* (Vol. 95, Issue 11, pp. 2167–2178). John Wiley and Sons Ltd. Doi:10.1002/jsfa.6993

Yang, B., Ahotupa, M., Maäättä, P., & Kallio, H. (2011). Composition and antioxidative activities of supercritical CO_2-extracted oils from seeds and soft parts of northern berries. *Food Research International*, *44*(7), 2009–2017. Doi:10.1016/j.foodres.2011.02.025

Yu, T., Lee, Y. J., Jang, H. J., Kim, A. R., Hong, S., Kim, T. W., Kim, M. Y., Lee, J., Lee, Y. G., & Cho, J. Y. (2011). Anti-inflammatory activity of Sorbus commixta water extract and its molecular inhibitory mechanism. *Journal of Ethnopharmacology*, *134*(2), 493–500. Doi:10.1016/j.jep.2010.12.032

Zahari, N. A. A. R., Chong, G. H., Abdullah, L. C., & Chua, B. L. (2020). Ultrasonic-assisted extraction (UAE) process on thymol concentration from Plectranthus amboinicus leaves: Kinetic modeling and optimization. *Processes*, *8*(3). Doi:10.3390/pr8030322

Zeb, A., & Murkovic, M. (2010). Analysis of triacylglycerols in refined edible oils by isocraticHPLC-ESI-MS. *European Journal of Lipid Science and Technology*, *112*, 844–851. Doi:10.1002/ejlt.201000064

Zhong, Y., & Shahidi, F. (2011). Lipophilized epigallocatechin gallate (EGCG) derivatives as novel antioxidants. *Journal of Agricultural and Food Chemistry*, *59*(12), 6526–6533. Doi:10.1021/jf201050j

Zolgharnein, J., Shahmoradi, A., & Ghasemi, J. B. (2013). Comparative study of Box-Behnken, central composite, and Doehlert matrix for multivariate optimization of Pb (II) adsorption onto Robinia tree leaves. *Journal of Chemometrics*, *27*(1–2), 12–20. Doi:10.1002/cem.2487

Zymone, K., Raudone, L., Raudonis, R., Marksa, M., Ivanauskas, L., & Janulis, V. (2018). Phytochemical profiling of fruit powders of twenty Sorbus L. Cultivars. *Molecules*, *23*(10), 1–17. Doi:10.3390/molecules23102593

SUMMARY IN ESTONIAN

Pihlaka (*Sorbus* spp.) genotüüpide viljade väärindamine funktsionaalse toidu koostisosadeks

Sissejuhatus

Viimastel aastakümnetel on teadlaste ülesandeks uurida ja katsetada uusi taimseid allikaid bioaktiivsete ühendite saamiseks. Need ühendid pakuvad huvi nii tervise- kui ka toitumisspetsialistidele. Paljusid taimi on veel vähe uuritud aspektist, kas nad sobivad antibakteriaalsete või -mikroobsete lisandite või antioksüdantidena funktsionaalse toidu, kosmeetikatoodete või ravimite koostisesse. Samuti tuleb hinnata taimse materjali töötlemisel tekkivate kõrvalsaaduste toiteväärtust ja uurida nende väärindamist funktsionaalseteks toidu koostisosadeks.

Kuigi roosõieliste sugukonda (*Rosaceae, Maloideae*) kuuluva pihlaka perekonna (*Sorbus* L.) kasvuala on lai ja sellesse perekonda kuulub üle 250 liigi, on genotüüpe vähe uuritud. Pihlaka viljad, kooreosa, lehed ja ka õied sisaldavad fütokemikaale, mida kasutatakse rahvameditsiinis (Robertson, *et al.*, 1991). Mitut pihlaka liiki on katsetatud 2. tüübi diabeedi ravis (Boath *et al.*, 2012; Broholm *et al.*, 2019; Termentzi, Alexiou, *et al.*, 2008). Pihlaka viljade ekstraktil on tõhus toime mitme bakteri (*Escherichia coli* , *Staphylococcus aureus*) kasvu ning laia spektriga mikroorganismide elutegevuse pärssimisel (Denev *et al.*, 2014; Mrkonjić *et al.*, 2017). Selle efekti annavad eelkõige pihlakas sisalduvad antioksüdantsete omadustega polüfenoolsed ühendid (fenoolhapped, antotsüaanid, flavonoidid) (Hukkanen *et al.*, 2006; Kylli *et al.*, 2010; Sołtys *et al.*, 2020).

Eestis kasvab looduslikult kolm liiki pihlakaid: harilik pihlakas (*S. aucuparia* L.), harilik tuhkpihlakas (*S. rupicola* (Syme) Hedl.) ja harilik pooppuu (*S. intermedia* (Ehrh.) Pers.)(Luuk, 2021). Neist tuntuim on Eestis laialt levinud harilik pihlakas (Luuk, 2021). Eestis kasvatatakse ka aretatud sorte ehk kultuurpihlakaid, mille fütokemikaalide sisaldus, maitse ja saagikus varieerub sorditi, kusjuures eriti väärtuslike omadustega on pihlaka hübriidsordid.

Vähene huvi pihlaka viljade kasutamise vastu toidus on eelkõige tingitud nende spetsiifilisest kootavast maitsest, mistõttu need ei sobi

nn lauamarjadeks. Pihlaka viljadest tehakse veini ja mahla, sealjuures tekib mahla valmistamisel märkimisväärne kogus pressjääki, millele ei ole praktilist rakendust. Samas näitavad uuringud, et pressjäägis sisaldub toidu funktsionaalseks lisandiks sobilikke fütokemikaale (Bobinaitė *et al.*, 2020; Jiang *et al.*, 2020; Tamkutė & Vaicekauskaitė, 2021). Pihlaka viljade seemned, kestad ja viljaliha sisaldavad näiteks vastavalt 60–70%, 28–35% ja 10% polüfenoolseid ühendeid (De Ancos *et al.*, 2015). Pressjäägist funktsionaalsete lisandite eraldamiseks on vajalik üksikasjalik teave viljade koostise ja antioksüdantsete omaduste kohta. Käesoleva uurimistöö käigus määrati 16 kultuurpihlaka sordi ja ühe looduslikult kasvava hariliku pihlaka viljade, mahla ja pressjäägi polüfenoolsete ühendite sisaldus ja vabade radikaalide sidumise võime ABTS•+, DPPH• ja ORAC meetodil.

Lisaks polüfenoolse koostise uurimisele valiti välja heade antioksüdatiivsete omadustega ja suure polüfenoolide sisaldusega sordid, mille vilju väärindati antioksüdantsete lisanditena kasutamiseks lihatoodetes. Lisanditega lihatooteid hinnati sensoorse, standardse ja metaboloomilise analüüsiga (Kerner *et al.*, 2021; Rocchetti *et al.*, 2020).

Eesmärgid

Uurimuse eesmärk oli selgitada välja Eesti Maaülikooli põllumajandus- ja keskkonnainstituudi aianduse õppetooli Polli aiandusuuringute keskuse kollektsioonaias kasvava 16 kultuurpihlaka sordi ja ühe looduslikult kasvava hariliku pihlaka hulgast suure polüfenoolide sisaldusega ja tugeva vabade radikaalide sidumisvõimega sordid nende pressjäägi väärindamiseks ja kasutamiseks rakendusuuringutes. Töö oli jagatud kolme etappi:

1) ülevaateartikli koostamine pihlaka (*Sorbus* L.) liikide keemilist koostist, antioksüdatiivseid omadusi, traditsioonilist, meditsiinilist ja toidulist kasutust kajastava teabe kaardistamiseks (artikkel **I**);

2) Polli aiandusuuringute keskuses saaki kandva 16 kultuurpihlakasordi ja sama piirkonna looduslikult kasvava hariliku pihlaka viljade, mahla ja pressjäägi polüfenoolsete ühendite sisalduse ja antioksüdantsete omaduste määramine (artikkel **II**);

3) kolme polüfenoolsete ühendite suurima sisaldusega ja parimate antioksüdantsete omadustega pihlakasordi viljade pressjäägi väärindamine kolmeks lihatoodete maitselisandiks ja nende lisandite hindamine lihamaatriksis (artikkel **III**).

Materjalid ja meetodid

Pihlaka viljades, mahlas ja pressjäägis polüfenoolsete ühendite sisalduse ja antioksüdantsete omaduste uurimiseks (**II**) koguti 2019. a Polli aiandusuuringute keskuse kollektsioonaias (58°21'N, 26°40'E, 68 m merepinnast) 16 viljakandvalt kultuurpihlaka sordilt ja samas piirkonnas looduslikult kasvava hariliku pihlaka taimedelt kokku 5 kg saaki. Viljad säilitati keskuse külmkambris -20 °C juures. Analüüsideks võeti igast sordist 1 kg vilju. Ülejäänud viljadest pressiti mahl aeglase mahlapressiga Smeg SJF01CREU (Smeg S.p.A, Guastalla, Itaalia). Viljade, mahla ja pressjäägi proovid külmkuivatati seadmega Advantage Plus Benchtop Freeze Dryer (SP Industries, Warminster, PA, USA) ja säilitati temperatuuril -40 °C. Seejärel jahvatati külmkuivatatud pressjäägi proovid veskis Retsch Mixer Mill M 400 (Haan, Saksamaa), segati mikrokristallilise tselluloosiga vahekorras 1:1 (w/w) ja valmistati kontsentratsioonid vahemikus 1–40 μg/mg. Külmkuivatatud mahla proovid lahustati metanoolis (MeOH, 1 w/v) ja töödeldi ultrahelivannis 15 minutit. Vilja- ja mahlaproove tsentrifuugiti enne annalüüsimist.

Artiklis **II** hinnati pihlaka viljade, mahla ja pressjäägi polüfenoolsete ühendite antioksüdantset toimet stabiilsete vabade radikaalide 2,2-difenüül-1-pikrüülhüdrasüüli (DPPH•9 ja 2,2'- asinobis 3-etüülbensotiasiliin-6-sulfonaadi(ABTS•+) sidumise meetodil ja hapniku radikaali absorbeerimisvõime alusel (ORAC) (Prior *et al.*, 2003; Re *et al.*, 1999). Mahla ja viljade 0,1%-lised ekstraktid koguses 7.5 μL (DPPH•) ja 3 μL (ABTS•+) segati FLUOstar Omega 96-süvikulises mikroplaadi lugejas analüüsimiseks vastavalt 300 μL DPPH• või ABTS•+ lahusega. Pressjääk või kontrolliks kasutatav tselluloos segati samuti mikroplaadilugejas 500 μL metanooli ja 1000 μL DPPH• lahusega või 25 μL metanooli ja 1500 μL ABTS•+ lahusega. Proovide absorptsiooni mõõdeti polüfenoolide reageerimisel DPPH• reagendiga võrdluses kontrolliga 515 nm juures või polüfenoolide reageerimisel ABTS•+ reagendiga võrdluses fosfaatpuhverdatud soolalahusega 734 nm juures. ORAC-analüüsil kasutati fluorestsentssondina fluorestseiini 75 mM fosfaatpuhvris (pH 7,4). Pihlaka viljade, mahla ja pressjäägi

ekstraktide lisamine pidurdas fluorestseerumise intensiivsuse langust, mis vastas proovi antioksüdantsusele. Kasutati 485 nm ergastus- ja 520 nm emissioonifiltreid. Kõigi kolme analüüsi tulemused väljendati μM Trolox equivalentides (TE)/g kuiva proovi kohta.

Polüfenoolide kogusisaldus määrati Folin-Ciocalteau reagendi redutseerumisel pihlaka fraktsioonidest pärinevate polüfenoolide abil, kasutades Singleton *et al.* (1999) meetodit. Absorptsiooni loeti lainepikkusel 765 nm. Tulemused avaldati mg GAE/g proovi kuivaine kohta.

Pihlaka viljade, mahla ja pressjäägi polüfenoolsete ühendite profiili määramiseks kasutati ülikõrge efektiivsusega vedelikkromatograafi koos mass-spektromeetriga (UHPLC-DAD-MS/MS). Selleks võeti 1 g värsket purustatud vilja, mahla või pressjääki ja lisati 10 mL 50% etanoolilahust, mis oli hapestatud 1% HCl-iga. Enne analüüsimist proovid homogeniseeriti kuulveskis IKA Ultra-Turrax® (Tube Drive, IKA®-Werke GmbH& Co. KG, Staufen, Saksamaa) 3 min kiirusel 6000 rpm. Seejärel töödeldi proove toatemperatuuril ultrahelivannis Branson 1800 (Emerson, St. Louis, MO, USA) 15 min ja loksutati 30 min multirotaatoris Multi RS-60 (Biosan Sia, Riia, Läti). Järgmisena proovid tsentrifuugiti kiirusel 13 000 rpm 10 min jooksul (Eppendorf MiniSpin, rotor F-45–13.11). Saadud ekstrakt (1 μL) pipeteeriti viaalidesse kvantitatiivseks ja kvalitatiivseks kromatograafiliseks analüüsiks. Polüfenoolide analüüs tehti kõrgsurvekromatograafiga UHPLC-DAD-LCMS 8040 (Shimadzu Nexera X2, Kyoto, Jaapan), kasutades pöördfaasi kolonni ACE Excel 3 C18-PFP, 100 mm × 2,1 mm (ACE® Advanced Chromatography Technologies Ltd., Aberdeen, Šotimaa) ja eelkolonni Security Guard ULTRA, C18 (Phenomenex, Torrance, CA, USA), töötades 40 °C juures. UHPLC-süsteem oli varustatud lahusti binaarse doseerimispumbaga LC-30AD, automaatse proovivõtjaga Sil-30AC, kolonni ahjuga CTO-20AC ning diood dioodrivi detektoriga SPD-M20A. Mobiilse faasi kiirus oli 0,25 mL/min ja süstitud proovi suurus 1 μL. Hapestatud (1% sipelghape) mobiilsed faasid koosnesid Milli-Q veest (A) ja metanoolist (B). Eraldamine viidi läbi 40 min jooksul järgmistel tingimustel: gradient 0–27 min, 10–80% B; 27–29 min, 80–95% B; 29–35 min, isokraatiline 95% B; süsteemi tasakaalustamine 10% B, 8 min enne järgmist süstimist. Kõiki proove hoiti analüüsi vältel 4 °C juures. Standardite kalibratsiooni vahemikud täpsustati arvestades eeldatavaid polüfenoolide kontsentratsioone proovides. Polüfenoolide identifitseerimiseks

võrreldi nende retentsiooniaegu ja UV-spektrit vastavate kirjandusest saadud tunnusainete retentsiooniaegadega. Mass-spektromeetriline andmeanalüüs viidi läbi LCMS 8040-ga, kusjuures elektropihustus-ionisatsiooniallikas (ESI) töötas nii positiivses kui negatiivses režiimis. Liidese pinge oli 4,5 kV (ESI+ ja ESI−). Nebuliseeriva (3 L/min) ja kuivatava (15 L/min) gaasina kasutati lämmastikku. Soojendusbloki temperatuur oli 350 °C ja desolvatiseerimisliinil (DL) 250 °C. Proove analüüsiti kolmes korduses. Tulemused esitati mg/g kuivaine kohta.

Artiklis **III** käsitleti sortide 'Likernaja', 'Solnetšnaja' ja hariliku pihlaka külmkuivatatud pressjäägisegust valmistatud lisandite kasutamist seahakklihapallides. Esimene lisand AC (500 g) saadi 1,8 kg pressjäägisegu rasvatustamisel ülekriitilise CO2 ekstraktori Separex 5 (Champigneulles, Prantsusmaa) abil temperatuuril 40 °C ja rõhul 40 MPa. Teise lisandi E jaoks võeti ülejäänud 1,3 kg rasvatustatud pressjääki ja tehti mikrolaine-ekstraktsioon 50%-lise etanooliga vahekorras 1:10 (w/v) 15 min võimsusel 300 W. Ekstraktsiooni lahus filtreeriti. Supernatandis olev etanool aurutati rotaatoraurustiga ja vesi eemaldati külmkuivatis VirTis Advantage Plus Benchtop Freeze Dryer Model XL-70 (SP Industries, Warminster, PA, USA). Selle meetodiga saadi teine lisand ehk ekstrakt E (292 g), mida hoiustati õhukindlalt pakendatuna temperatuuril -32 °C. Filtratsioonijääk külmkuivatati ja sellest sai kolmas lisand R (850 g).

Kõigi lisandite polüfenoolide üldsisaldus määrati Folin-Ciocalteau meetodil. Lisandid AC, E ja R segati kontsentratsioonides (1%, 2%, 3% ja 5%) vastavalt retseptile hakkliha, vee ja soolaga. Valmistati ka ilma lisanditeta kontrollproov (88% hakkliha, 11% vett, 1% soola) (**III**). Kaalutud lihapallid küpsetati ahjus (Inoxtrend E1CUA-107E, Santa Lucia di Piave, Itaalia) 145 °C juures 15 minutit. Jahtunud lihapallid kaaluti ja arvutati küpsetuskadu. Valmistamispäeval analüüsiti ka lihapallide keemilist koostist (niiskus, valk, rasv, tuhk). Küpsetatud lihapalle hinnati sensoorselt, et leida kõige aktsepteeritavama maitsega variandid. Lihapallide füüsikalis-keemilisi parameetreid (vee aktiivsus, pH ja värvus L*a*b* skaala järgi) hinnati valmistamise päeval ja 5. päeval. Edasisteks analüüsideks pakendati lihapallid valmistamise päeval modifitseeritud atmosfääriga (70% N2 ja 30% CO2) pakenditesse ning säilitati 4 °C juures. Valituks osutunud lihapallide lisandite (2%-AC, 2%-R ja 1%-E) vabade radikaalide (DPPH•) neutraliseerimisvõimet analüüsiti lihapalli maatriksis ja võrreldi kontrollproovi vastava tulemusega.

Lihapallide metaboloomika teostati säilituse kolmes ajapunktis (päevadel 0, 4, 14), et määrata säilitamise käigus tekkivad madalmolekulaarsed ühendid (**III**). Metaboloomika jaoks ekstraheeriti 1 g külmkuivatatud lihapalle 10 mL 80%-lise metanooli (v/v) lahusega, millele oli lisatud 0,1% (v/v) sipelghapet (mõlemad LC-MS kvaliteediga, VWR, Milaano, Itaalia). Segu töödeldi Ultra-Turrax kuulveskis (Ika T10, Staufen, Saksamaa) 5 min jooksul, tsentrifuugiti (Eppendorf 5810R, Hamburg, Saksamaa) (7800 x g) 15 min 4 °C juures ja filtreeriti läbi 0,22 µm tselluloosist süstlafiltri viaalidesse instrumentaalseks analüüsiks. Sihitamata profiilianalüüsi jaoks kasutati Q-ExactiveTM Focus hübriidkvadrupoolil Quadrupole-Orbitrap põhinevat kõrglahutusega massispektromeetrit (Thermo Scientific, Waltham, MA, USA), mis oli ühendatud Vanquishi ülikõrgsurvevedelikkromatograafi (UHPLC) pumbaga ja varustatud kuumutatud elektropihustusionisatsiooni (HESI)-II sondiga (Thermo Scientific, USA).

Artiklis **III** kasutati kromatograafiliseks eraldamiseks atsetonitriili vesilahust (6- 94% 35 minuti jooksul) liikuva faasina, faasi modifikaatorina kasutati 0,1% sipelghapet, kasutades BEH C18 (2,1x100 mm, 1,7 μm) kolonni 35 °C juures. Süstimismaht oli 6 µL ja elueerimise voolukiirus 200 µl/min. Täieliku skaneerimise mass-spektromeetriline analüüs viidi läbi positiivse ionisatsiooni režiimis massi eraldusvõimega 70 000 FWHM m/z 200 juures. Süstimisjärjestus randomiseeriti, igat proovi tehti kolm korda. HESI ja MS-DIAL-i parameetrid olid eelnevalt Rocchetti *et al.* (2021) poolt optimeeritud. Identifitseerimisetapp põhines massi *täpsusel, isotoopmustril (st isotoopide jaotusel, ruumil ja arvukusel) ja spektri sobivusel.* Kogu tuvastamise piirskooriks määrati 50%. Liha metaboliidid annoteeriti FooDB andmebaasi abil. Annoteerimata massiühendite fragmenteerimiseks kasutati MS-Finder arvutitarkvara ning FooDB ja Lipid Mapsi teeke. Säilitati ühendid, mille arvuti ennustusskoor oli kõrgem kui 5.

Tulemused

Pihlakasortide (vilja, mahla ja pressjäägi) polüfenoolsete ühendite sisaldus (II)

Polüfenoolide üldsisaldus oli 16 kultuurpihlaka sordi ja hariliku pihlaka viljades ja mahlas väiksem kui pressjäägis. Rohkem polüfenoole oli hübriidsortides 'Likernaja', 'Burka', 'Rubinovaja' ja 'Granatnaja'. Mitte-

hübriidsortidest eristusid polüfenoolide suure sisalduse poolest määri pihlakas, 'Solnetšnaja' ja looduslik pihlakas.

Vabade radikaalide sidumise võime (II)

DPPH• neutraliseerimise aktiivsus oli pihlaka viljadel ja mahlal väiksem kui pressjäägil. Antioksüdatiivsuse analüüside DPPH•, ABTS•+ ja ORAC puhul ületasid hübriidsortide 'Likernaja', 'Burka', 'Rubinovaja' ja 'Granatnaja' analüüside arvulised väärtused 16 kultuurpihlaka sordi ja hariliku pihlaka keskmist väärtust. Antioksüdatiivne toime oli kõige suurem hübriidsortide pressjääkides. 'Solnetšnaja' sordi kõigil fraktsioonidel (marjal, mahlal ja pressjäägil) olid ORAC-i väärtused ja samuti pressjäägi DPPH• ja ABTS•+ väärtused suuremad kui 16 kultuurpihlaka sordi ja hariliku pihlaka keskmine. Kui DPPH• keskmised väärtused tõusevad järjestuses mahl < vili < pressjääk, siis ORAC-i ja ABTS•+ väärtused järjestuses pressjääk < mahl < vili.

Polüfenoolsete ühendite identifitseerimine ja kvantifitseerimine (II)

Kõrgsurvekromatograafilise analüüsi tulemused näitasid, et pihlaka sordid sisaldavad rohkelt neoklorogeen- ja klorogeenhappeid. Neoklorogeenhappe sisaldus on kõige suurem hübriidsortide mahlas. Suur klorogeenhappesisaldus tuvastati sortide 'Sahharnaja', 'Bussinka', 'Angri' ja loodusliku pihlaka mahlaproovides ning sortide 'Bussinka' ja 'Sahharnaja' ning loodusliku pihlaka pressjääkide proovides. Neoklorogeen- ja klorogeenhapped olid ka pressjäägiproovides kõige domineerivamad fenoolsed happed.

Suurim antotsüaniinide (ACY) kontsentratsioon oli hübriidsortide 'Burka', 'Likernaja', 'Granatnaja' ja 'Rubinovaja' viljades. Vilja- ja mahlaproovides olid kõige domineerivamad antotsüaniinid tsüanidiin 3-O-galaktosiid ('Rubinovaja' proovides kuni 91%) ja tsüanidiin-3-arabinosiid ('Likernaja' ja 'Burka' proovides kuni 21–22%). Pressjäägi proovides leidus antotsüaniinidest kõige rohkem tsüanidiin-3-glükosiidi (kuni 97%). Antotsüaanide keskmine väärtus pressjäägi proovides oli kuni 10 korda madalam kui vilja- ja mahlaproovides. Samas oli flavanoolide sisaldus pressjäägi proovides kuni 4,8 korda suurem kui vilja- ja mahlaproovides.

Antioksüdantsete omaduste, polüfenoolide sisalduse ja kahe aasta (2019 ja 2021) saagikuse tulemuste põhjal valiti välja sordid edasisteks rakendusuuringuteks. Parimad näitajad olid hübriidsortidel 'Likernaja' ja 'Burka', kultuurpihlaka sortidel 'Sahharnaja' ja 'Solnechnaja' ning looduslikul pihlakal.

Lihapallide koostises olevate pihlaka pressjääkidest lisandite sihitamata metaboloomika ja kvaliteet (III)

Esialgsetest analüüsidest selgus, et lisandi E polüfenoolide sisaldus oli AC-ga võrreldes ligikaudu 5 korda ning R-i omast 17 korda suurem.

Siinses uurimistöös kasutatud hakkliha sisaldas 67,43% niiskust, 18,49% valku, 13,85% rasva ja 0,96% tuhka. Lihapallide valmistamiseks lisati hakklihale 11% vett ja 1% soola ning pressjäägist valmistatud erinevas kontsentratsioonis (1–5%) lisandeid. Sensoorse hindamisega määrati iga lisandi puhul sobivaim kontsentratsioon. Edasisteks katseteks valiti igast grupist sensoorselt parim (2%-AC, 1%-E ja 2%-R), samuti ilma lisandita kontrollproov. Vaba radikaali (DPPH•) neutraliseerimise katses andsid lisanditega lihapallid võrreldes kontrollprooviga 1%-E puhul 15 korda, 2%-AC puhul 10 korda ja 2%- R puhul 5 korda paremaid tulemusi.

Madala kontsentratsiooni (1–2%) tõttu ei olnud taimsete lisanditega lihapallidel niiskuse-, tuha- ja valgusisalduses olulist erinevust, küll aga vähendasid kiudainerikkad AC ja R lisandid lihapallide rasvasisaldust ning lisand R vähendas küpsetuskadu rohkem kui 13%. Lisand R suurendas lihapallide mahlasust kontrollprooviga võrreldes rohkem kui 2%, lisand E aga suurendas oluliselt küpsetuskadu.

Pärast 5-päevast säilitamist (4 °C) oli 1%-E lisandiga lihapallide pH märkimisväärselt madalam kui kontrollproovil, mis on selgitatav E lisandi klorogeenhappe sisaldusega. Lisandeid 2%-AC ja 2%-R sisaldavate proovide pH püsis 5-päevasel säilitamisel stabiilsena, aga kontrollproovi pH väärtus tõusis, viidates liha mikrobioloogilisele riknemisele.

Lihapallide värvuse analüüsil ja L*a*b* väärtuste määramisel selgus, et suure antotsüaniinide sisalduse tõttu vähendasid pressjäägi lisandid liha heledust (L*) ja suurendasid punasust (a*) kuni 48%. Bioaktiivseid ühendeid sisaldavad lisandid 1%-E ja 2%-AC vähendasid lihapallide kollasust (b*) vastavalt 1,87% ja 0,42%.

Erinevate lisanditega lihapallide sihitamata UHPLC-Orbitrap-i analüüsi käigus annoteeriti kolmes säilitusaja punktis 402 ühendit vastavalt nende individuaalsetele massi spektritele ja isotoopide profiilile. (lisamaterial **III**). Proovide rühmitamiseks vastavalt nende keemilise profiili olemuslikele sarnasustele kasutati hierarhilist klasteranalüüsi (HCA) ja koostati vastav soojuskaart. HCA koosnes kahest klastrist, millest esimene hõlmas hierarhiliselt kontrollproove säilitusajapunktides 0, 4 ja 14 päeva, ning teine näitas lisanditega lihapalle samades ajapunktides. Nii soojuskaart kui ka sihitamata peakomponentanalüüsi (PCA) graafiku esimene peakomponent (PC1) näitasid pressjäägist saadud lisandite ja säilitusaja võimalikku mõju liha metaboolsele ja keemilisele profiilile. Säilitusaja mõju täiendavaks uurimiseks lihapallide proovide keemilisele profiilile koos lisanditega ja ilma lisandita kasutati ortogonaalse projektsiooni varjatud struktuuride eristamise analüüsi (OPLS-DA). OPLS-DA graafikute ennustusmudelid näitasid selget eristumistrendi säilimisaja erinevate punktide vahel, samuti väga häid sobitumise ($R2Y > 0,9$) ja prognoosimisvõime ($Q2 > 0,5$) väärtusi. Seejärel hinnati liha metaboliitide muutusi kogu säilivusaja jooksul (14 päeva), et mõista oksüdatiivsete protsesside võimalikku mõju lihapallide koostisosadele ning pressjäägist saadud lisandite kaitsvat toimet lihapallidele. Seetõttu koostati OPLS-DA mudel, et paremini mõista, millised metaboliidid tekkisid lihapallide proovides 14. säilituspäevaks. OPLS-DA graafik näitas, et kontrollproov (C14) eraldus lisanditega proovidest mööda ortogonaalset vektorit. Seejärel eristati OPLS-DA mudeli abil 184 metaboliiti. Need markerühendid on loetletud tabelis S1 (lisamaterjal **III**) rühmitatuna andmebaasis FooDB toodud keemiliste ühendite klassidesse. Üldiselt eristusid kõige enam terpenoidid (52 ühendit), aminohapped (26 ühendit), rasvhapete derivaadid (sh estrid, happed ja alkoholid), polüfenoolid (16 ühendit) ning mõned aldehüüdid ja ketoonid. Rasvhapete derivaadid, aldehüüdid ja ketoonid esinesid kontrollproovis, kuid mitte lisanditega proovides (Tabel S1, lisamaterjal **III**). Selgus, et 1%-E pärssis aldehüüdide ja ketoonide teket 14-päevase säilivusaja jooksul kõige efektiivsemalt. Lipiidide oksüdatsiooni saab tuvastada kõrvalmaitse ja lõhna järgi, mille tekitab karbonüülühendite lenduv fraktsioon. Artikli **III** lisamaterjali põhjal leidus lihapallides viis eristuvat aldehüüdiühendit, nende hulgas 7-dodecenaal ja 2,4-heptadienaal. Samuti vähenes 14-päevase säilitusaja jooksul linoolhappe derivaatide, terpenoidide (sh mono-, di-, tri-, tetra- ja seskviterpenoide) ja fenoolühendite (peamiselt flavonoidide ja fenoolhapete) sisaldus kontrollproovis (C), mis viitab suuremale lipiidide peroksüdatsioonile, võrreldes lisanditega lihapallidega. Klorogeenhape

ja isokvertsitriin olid lihapallides kõige levinumad pihlakaga seonduvad tuvastatud polüfenoolsed ühendid. Lisanditega seondusid ka mõned olulised triterpenoidid, nagu 3-trans-p-kumaroüülrotundhape (mustika biomarker) ja põletikuvastaste omadustega glütsürretiinhape. Nende ühendite akumuleerumine lisanditega lihapallides kõlblikkusaja lõpul ja karbonüülühendite puudumine näitavad, et lisandid võivad kaitsta lipiidide oksüdatsiooni eest.

Järeldused

Doktoritöös uuriti *Sorbus* L. perekonda ja koostati ülevaateartikkel pihlaka genotüüpide olulisematest aspektidest. Seejärel uuriti 16 Eestis kasvatatud kultuurpihlaka hübriidi ja sordi ning looduslikult kasvava hariliku pihlaka viljade, mahla ja pressjäägi antioksüdantsust ja polüfenoolset koostist, et leida kõige suurema polüfenoolide sisalduse ning antioksüdantsusega sordid. Valitud sortide pressjääke töödeldi vastavalt katseplaanile ja neid kasutati toidu funktsionaalse koostisosana.

Püstitatud hüpoteesidele ja eesmärkidele tuginedes on käesoleva uurimuse järeldused järgmised:

- Hüpotees, et märkimisväärne osa suure antioksüdatiivsusega fütokemikaale ja polüfenoole jääb pihlaka viljade pressjääki, sai täielikult tõestatud (**II**) ja see fraktsioon võib olla potentsiaalseks funktsionaalsete lisandite allikaks biorafineerimise protsessis, et suurendada pihlaka sortide kasutust.

- Uurimistöö käigus tuvastati suurima polüfenoolide sisalduse ja parimate antioksüdantsete omadustega sordid pihlaka viljade edasiseks väärindamiseks. Nendeks sortideks olid kõik hübriidsordid: 'Likernaja', 'Burka', 'Granatnaja' ja 'Rubinovaja', mõned *S. aucuparia* L. seemikud: sordid 'Solnechnaja' ja 'Sahharnaja', ja harilik pihlakas *S. aucuparia* L.

- Pihlaka viljade pressjäägi väärindamise tulemusel saadi looduslikud antioksüdantsed lisandid lihapallidele: rasvatustatud pressjääk, ekstraktsioonijääk ja külmkuivatatud etanooliekstrakt. Kolmest lisandist oli kõige efektiivsem külmkuivatatud etanooliekstrakt. Pressjäägist saadud lisandid takistasid lihapallide säilituskatse ajal 14 päeva jooksul karbonüülühenditest põhjustatud

ebameeldiva kõrvalmaitse ja lõhna teket. Linoolhappe derivaatide kontsentratsioon vähenes katse jooksul ainult kontrollproovis. Saadud tulemused tõestavad hüpoteesi, et pihlaka viljade pressjäägist valmistatud lisandid võivad olla funktsionaalse toidu kasulikud koostisosad, mis kaitsevad lihatooteid oküdatsiooni eest (**III**).

Edasistes uuringutes tuleb keskenduda järgmistele aspektidele:

- pihlaka viljade pressjääkidest SFE-CO2 lipofiilsete ühendite eraldamine, kasutades kaaslahustina etanooli, et kontsentreerida väärtuslikke lipofiilseid ühendeid nagu β-karoteen, α-, β- ja γ-tokoferool ja fütosteroole nagu sitosterool, β-stigmasterool ja kampesterool ning lenduvaid ühendeid;

- fraktsioneerimistingimuste (aeg, rõhk, temperatuur, kaaslahusti kontsentratsioon) optimeerimine iga lipofiilse komponendi (β-karoteen, fütosteroolid, tokoferoolid, lenduvad ühendid) jaoks eraldi, et saada suuremaid saagiseid ja kõrgemaid kontsentratsioone nende komponentide kasutamiseks kosmeetikatoodetes ja toidulisandites;

- keskkonnasõbralike ja kulutõhusate ekstraheerimismeetodite väljaselgitamine, et saada suurema saagise ja kõrge polüfenoolide sisaldusega ekstrakte, mis pärsivad toidu oksüdatsiooni;

- pihlaka ja muude puuviljade töötlemisel tekkinud kõrvalsaaduste ekstraktide või pulbrite laialdasemate kasutusvõimaluste leidmine;

- pihlaka viljade pressjäägi lisandite antioksüdatiivse ja sensoorse mõju statistiline võrdlemine sagedamini kasutatava taimse lisandiga (küüslauk, rosmariin, pune, sibul, pipar), et hinnata pressjäägi kasutamisvõimalusi toidus.

ACKNOWLEDGEMENTS

The present study was accomplished at the Estonian University of Life Sciences under the supervision of Prof. Petras Rimantas Venskutonis (Kaunas University of Technology, Department of Food Science and Technology), Prof. Rajeev Bhat (Estonian University of Life Sciences, ERA Chair for Food (By-) Products Valorisation Technologies (VALORTECH) and Dr. Reelika Rätsep (Estonian University of Life Sciences, Polli Horticultural Research Centre, Institute of Agricultural and Environmental Sciences). I am very grateful for their contributions to my thesis. They have supported me throughout the years of the study.

This work contributed to the ERA Chair in VALORTECH project, which has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 810630.

This work also contributed to the Plantvalor Project "PlantValor–full-scale product development service in synergy with the traditional activities of Polli Horticultural Research Centre 2020–2023", which has received funding from the European Regional Development Fund.

I

ORIGINAL PUBLICATIONS

Sarv, V., Venskutonis, P.R., Bhat, B. 2020. The *Sorbus* spp.—Underutilised Plants for Foods and Nutraceuticals: Review on Polyphenolic Phytochemicals and Antioxidant Potential. *Antioxidants* 9, 813: 1-23. doi:10.3390/antiox9090813

antioxidants

MDPI

Review

The *Sorbus* spp.—Underutilised Plants for Foods and Nutraceuticals: Review on Polyphenolic Phytochemicals and Antioxidant Potential

Viive Sarv [1,2,*], Petras Rimantas Venskutonis [1,3] and Rajeev Bhat [1]

[1] ERA Chair for Food (By-) Products Valorisation Technologies of Estonian University of Life Sciences -VALORTECH, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1a, 51014 Tartu, Estonia; rimas.venskutonis@ktu.lt (P.R.V.); rajeev.bhat@emu.ee (R.B.)

[2] Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre, 69108 Polli, Estonia

[3] Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, LT-50254 Kaunas, Lithuania

* Correspondence: viive.sarv@emu.ee

Received: 25 July 2020; Accepted: 26 August 2020; Published: 1 September 2020

Abstract: The *Sorbus* spp. are valuable plants, which have been used for ornamental purposes, in traditional medicines and less seldom in foods. Recent studies have revealed different anatomical parts of the *Sorbus* spp. to contain valuable phytochemicals demonstrating various bioactivities. However, in terms of applications in the products intended for human consumption, *Sorbus* still remains as an underutilised genus. The increasing number of studies on phytochemicals, antioxidant potential and other bioactivities of *Sorbus* extracts has revealed the prospects of expanding its use in natural medicines, cosmetics and as innovative food ingredients, which might find wider applications in functional foods and/or nutraceuticals. Caffeoylquinic acids, flavonoids and proanthocyanidins have been reported in various *Sorbus* spp. as the most abundant polyphenolic antioxidants. The preparations of various plant anatomical parts have been used in ethnopharmacology as natural remedy for treating bacterial, viral, inflammatory diseases including tumors. *Sorbus* spp. plant parts have also been tested for management of diabetes, neurological, and cardiovascular disorders. The present review is focused on *Sorbus* plants (in total 27 *Sorbus* spp.), their composition and properties in terms of developing promising ingredients for foods, nutraceutical, cosmeceutical and other applications. It is expected that this review will assist in designing further studies of rowans and other *Sorbus* spp. in order to expand their uses for various human applications.

Keywords: rowan; phytochemical composition; bioactivities; health benefits; food applications

1. Introduction

During the past few decades, search and development for novel highly valued bioactive compounds from plants has become a topical issue for researchers, health professionals, producers, and consumers. Considering vast number of species in the Plant Kingdom, there are still infinite number of under explored plants, which may serve as an excellent platform for discovery of new compounds and developing valuable preparations. Underutilised plants have become of a particular interest in the era of functional foods, nutraceuticals and personalized nutrition. Thus, natural bioactive compounds can play the most important role in the development of health promoting products based on individual genome and/or microbiome [1,2].

Fruits and vegetables have been considered as healthy foods, mainly owed to the presence of high amounts of valuable nutrients such as vitamins, minerals, polyphenolic antioxidants, dietary fibre and others. In this regard, many well-known comprehensively valorised and globally commercialized

berry fruits such as raspberries, strawberries, black currants, blueberries, cherries and others are among the richest sources of vitamins and bioactive phytochemicals, particularly antioxidant polyphenols. The above-mentioned berries also possess characteristic and highly appreciated sensory properties and hence are consumed both as fresh fruits and/or in processed forms. However, there are still many underutilised berries, mainly due to their specific and therefore non-acceptable for consumers flavour.

The *Sorbus* spp. (common names rowans, whitebeams and others) are deciduous shrubs or trees, which although being widely grown in the gardens and parks, can be assigned to the underutilized plants in terms of their applications as foods, nutraceuticals and/or cosmeceuticals. The rowans are the most widely studied *Sorbus* spp. Wild rowan trees are tolerant to harsh Nordic climate and poor growing environment such as rocky and windy slopes and even the mountains and may reach up to 15 m height.

Other anatomical parts of berry producing plants may also contain valuable phytochemicals; therefore, bark, leaves, inflorescences have been empirically used in folk medicines for centuries. The bark of the *Sorbus* trees is mostly smooth, lustrous, dark, with elongated horizontal lenticels; the leaves are pinnately compound, the leaflets toothed or rarely entire, while the inflorescences may be extra-large, convex panicles [3]. The interest in *Sorbus* spp. as a promising source of valuable phytochemicals has increased during last decade. For instance, in the Clavirate Analytics Web of Science database, out of 133 publications with the search words '*Sorbus* + antioxidants' 105 have been included since 2010; while in the same period 68 records out of 91 have been found with the search words '*Sorbus* + polyphenolics' (accessed on 20 July 2020). Comprehensive review on *Sorbus* phytochemicals has been recently published; it focuses on *Sorbus* as an ethnopharmacologically important but underestimated genus and provides extensive information on plant phytochemicals [4]. The present review focuses on *Sorbus* composition and properties in terms of development of promising ingredients for food, nutraceutical, cosmeceutical and other applications. For this purpose, it includes some important information; for instance, more detailed data on until now reported concentrations of different polyphenolic phytochemicals and the values of antioxidant potential in different *Sorbus* spp. This information might assist in selecting the most promising species/cultivars and their anatomical parts for further studies and applications.

2. Botanical Classification and General Uses

The copious genus *Sorbus* L. (Rosaceae, Maloideae) covers up to 250 species, which in addition are divided into 6 subgenus, namely *Sorbus*, *Aria*, *Micromeles*, *Cormus*, *Tominaria*, and *Chamaemespilus*. According to Robertson et al. [3] approximately 35 species exist in the Caucasus and Turkey, 91 in Europe, and 111 in China, Vietnam, Myanmar, and in the Himalayas. The bitter fruits of wild rowan are round in shape and they can be red, orange, yellow, pink or white with homogeneous flesh (Figure 1) [3]. The rowan tree can yield up to 20 kg of rowanberries [5]. Traditionally, people consumed rowanberries in small amounts as a mash to improve the appetite and stimulate production of gastric acid. In folk medicine these fruits have been used as a laxative, against rheumatism and kidney diseases, and gargle juice against hoarseness [6]. Rowan berries have been traditional diuretic, vasodilatory, anti-inflammatory, anti-diarrheal remedies and a source of ascorbic acid (vitamin C); in some countries they also have been used for treating intestinal obstructions, various liver and gallbladder diseases [7]. The leaves have sometimes been used to feed livestock while the fruits have been administered to domestic pigs and goats against bacterial infections [8]. In order to make the selection of abundant genus *Sorbus* the species listed in the United States Department of Agriculture (USDA) database [9] were used in the current review. In addition, the species with a more comprehensively investigated bioactivity were included.

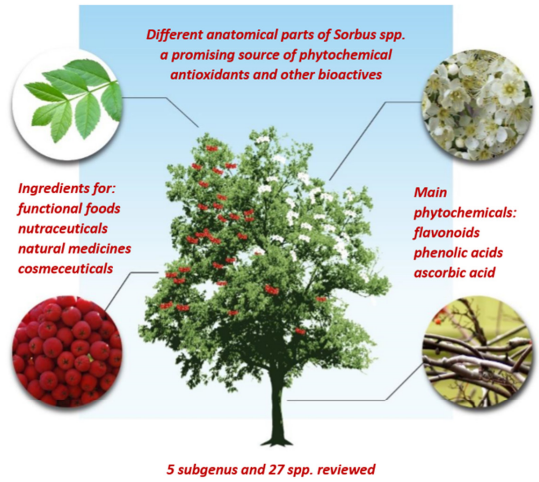


Figure 1. Potential uses of different parts of *Sorbus* spp.

The subgenus *Sorbus*, commonly noted as a mountain ash (Amur or European mountain ash), rowan or quick beam, is distributed in the Northern Hemisphere. It has hairless or thinly hairy leaves [3]. This review covers 19 species from the large *Sorbus* subgenus (Table 1): *S. americana* Marshall (American mountain ash), *S. aucuparia, S. californica* Greene (California mountain ash), *S. cashmiriana* Hedl., *S. commixta* Hedl. (The Japanese rowan), *S. decora* C.K. Schneid, (the northern mountain ash), *S. dumosa* Greene (Arizona Mountain Ash), *S. gracilis* (Sieb. & Zucc.) K. Koch., *S. groenlandica* (C.K. Schneid.) A. Löve & D. Löve (the Greenland mountain-ash), *S. koehneana* C.K. Schneid. (Koehne mountain ash), *S. pohuashanensis* (Hance) Hedl., *S. pogonopetala* Koehne, *S. sambucifolia* (Cham. & Schlecht.) Roem. (Siberian Mountain-ash), *S. scalaris* Koehne, *S. scopulina* Greene, *S. setschwanensis* (C.K. Schneid.) Koehne, *S. sitchensis* M. Roem (western mountain ash), *S. tianschanica* Rupr., *S. wilfordii* Koehne. Different anatomical parts of these species have been used for medicinal and food purposes (Figure 1). The leaves of *S. tianschanica* have been used to treat asthma, ventricular myocytes, dyspnoea, tuberculosis and gastritis [10], while both the leaves and the bark of *S. decora* are known as an antidiabetic medicine [11]. The bark of *S. americana,* due to hypo-glycaemic properties has also been used for treating diabetes; while other applications include vaso-relaxant, antitussive and tonic activities [12]. In oriental medicine, the stems and bark of *S. commixta* have been used to treat arthritis and inflammatory diseases and as hypoglycaemic, vasorelaxant, antitussive and tonic agents [13,14]. The bark preparation of *S. cashmiriana* has been used to treat nausea and heart diseases, while its berries have been used to cure scurvy [15]. The fruits, stems and bark *S. pohuashanensis* have been widely used in traditional Chinese medicine for treating chronic tracheitis, tuberculosis and oedema [16]. The fruits of *S. sambucifolia* have been used in drinks and foods (beverages, jams, jellies, floured dried fruit, etc.), while for medicinal purposes—in case of avitaminosis, arteriosclerosis and as antipyretic or diuretic agent. Indigenous people used to eat fresh *S. scopulina* berries; however, currently they are sometimes used in pies, preserves, or wine-making [17]. In folk medicine, the fruits and the inflorescences of *S. aucuparia* (European rowan) have been used as traditional anti-inflammatory, antidiarrheal, vasodilatory and an appetite-improving agents, as well as a good source of vitamins, diuretic and mild laxative medicine [18,19]. In traditional Austrian medicine, the tea, syrup, jelly or alcoholic tincture of *S. aucuparia* fruits have been used to treat fever, infections, colds, flu, rheumatism and gout [20].

From the subgenus *Aria* with 39 species, commonly known as whitebeams, 6 species, namely *S. aria* Crantz, *S. intermedia* (Ehrh.) Pers., *S. norvegica* Hedl., *S. folgneri* (Schneid.) Rehd., *S latifolia* (Lam.) Pers, *and S. minima* (Ley) Hedl. are covered in this review. These species have simple white-hairy leaves and are distributed in the temperate regions of Europe and in Asia. Traditionally, the leaves of *S. aria* were consumed as antidiarrheal ingredients, while their berries have been used in jellies, jams, brandy, liqueurs, conserves and vinegar, as traditional bread flour extender, diuretic, anti-inflammatory, anti-diarrhoeal, vasodilatory agent and vitamin source [21]. Moreover, the fruits and inflorescences of *S. aria* have been used as a diuretic, laxative and emmenagogue folk medicine for treating painful menstruation, constipation and kidney disorders [22]. The berries of *S. intermedia* have been added to bread in Estonia [23], while the berries of *S. norvegica, S. folgneri, S latifolia* and *S. minima* were tested for their α-amylase and α-glucosidase inhibitory activities [24].

The subgenus *Micromeles*, commonly known as Korean whitebeam, alder-leafed whitebeam, contains around 25 narrow leaved species of shrubs and trees with white flowers, distributed from Nepal to the South Kuriles, extending to the Malay Peninsula and Sumatra [25]. In the Korean folk medicine the twigs of the most widely distributed species, *S. alnifolia* (Siebold & Zucc.) K. Koch., were used for treating neurological disorders [26].

Aria, *Micromeles* and *Chamaemespilus* have simple leaves and pomes with groups of tanniferous cells, however, *Chamaemespilus* (false medlar or dwarf whitebeam) differs by a rather different flower shape [27]. *Chamaemespilus* is not reviewed in the current study due to the lack of information about its uses and antioxidant activity.

The subgenus *Torminaria* (common names wild service tree, chequers, and checker tree) with three species is distributed in the temperate Europe, south to the mountains of North Africa and east to the Caucasus ranges. It has maple-like simple, 3-5-lobed leaves and brown pomes without groups of tanniferous cells. The fruits of *S. torminalis* have been traditionally used as diuretic or anti-inflammatory, antidiarrheal (dried), vasodilatory remedy and as a source of vitamins [27,28]. In the current work, the uses and antioxidant potential of 2 varieties of *S. torminalis*, (var. *torminalis* and *semitorminalis*) are surveyed.

The subgenus *Cormus* with pomes without starch and groups of tanniferous cells [27] and compound leaves, is distributed in the warm-temperate Europe, North Africa and Asia. Unlike the subgenus *Sorbus*, *Aria* and *Torminaria*, whose fruit carpels are not fused, subgenus *Cormus* is with distinct fused carpels in the fruit. In this review only *S. domestica*, also known as true service tree or sorb tree [29], is included. It has been reported that the fruits of *S. domestica* are traditional anti-inflammatory, antidiarrheal (dried), antidiabetic, diuretic, vasodilatory agents and vitamin source [30].

Although, wild rowanberries are sour in taste they still contain a wide array of healthy components. In the beginning of the 20th century, the Russian practitioner Michurin started the breeding program with *S. aucuparia* to improve the flavour and increase the fruit mass of rowanberries. Crossbreeding of rowan with the *Malus*, *Mespilus*, *Aronia*, or *Pyrus* spp. produced interesting sweet-fruited rowan hybrids. These new hybrids have been bred particularly for northern conditions and they have demonstrated great frost-resistance in the Nordic countries [31]. The famous crossbreeds of *S. aucuparia* in Russia were called 'Burka', 'Likjornaja', 'Dessertnaja', 'Granatnaja', 'Rubinovaja', and 'Titan' [32]. The Western European hybrids of *S. aucuparia* include 'Apricot Queen', 'Brilliant Yellow', 'Chamois Glow', 'Pink Queen', and 'Salmon Queen' [33]. In contrast to wild rowanberries, the hybrids are much more palatable [34] and the sugar content in their cultivars is 1.2–2.1 times higher than in the wild rowanberries [35]. In the current review, the bioactivity and phytochemical contents of several *S. aucuparia* cultivars are compared with the wild berries.

Table 1. Botanical classification of selected *Sorbus* spp. and their uses.

Species and Varieties (Subgenus)	Food Uses of Fruits	Anatomical Part: Medicinal and Other Uses	Ref.
S. alnifolia (Sieb. & Zucc.) K. Koch. (*Aria*)		Twigs: treatment of neurological disorders as a traditional medicine in Korea	[26]
S. americana Marshall—American mountain ash (*Sorbus*; informal group *Commixtae*)		Bark: treatment diabetes hypo-glycaemic, vaso-relaxant, antitussive and tonic agent	[36]
S. aria L. Crantz—chess-apple (*Aria*)	Jellies, jams, brandy, liqueurs, conserves and vinegar, traditional bread flour extender	Fruit: diuretic, anti-inflammatory, anti-diarrhoeal, vasodilatory and vitamin agent; leaves: ethnomedical antidiarrheal ingredients; inflorescences and fruit: diuretic, laxative and emmenagogue; treatment of painful menstruation, constipation and kidney disorders	[11] [18] [37]
S. aucuparia L.—European mountain ash (*Sorbus*)	Alcohol beverages, jams, jellies, honey (floured dried fruit)	Traditional diuretic, anti-inflammatory, antidiarrheal (dried fruits), vasodilatory and an appetite-improving agent, source of vitamins, mild laxative	[18] [22] [7]
S. cashmiriana Hedl. (*Sorbus* series Multijugae)		Bark: tea made from its bark—to treat nausea, the bark preparation- to treat heart diseases; berries: to cure scurvy	[15]
S. commixta Hedl. (*Sorbus*; informal group *Commixtae*)		Stembark: for treating asthma, bronchitis, gastritis and oedema, anti-inflammatory, -atherosclerotic, -alcoholic, and vascular-relaxant effects, anti-atherogenic, for treating arthritis, hypoglycaemic, antitussive and tonic agent	[38] [14] [39] [13]
S. decora (Sarg.) C.K. Schneid—northern mountain ash (*Sorbus*; informal group *Commixtae*)		Leaves and bark- an antidiabetic medicine	[11]
S. domestica L. (*Cormus*)	Food ingredients	Traditional diuretic, anti-inflammatory, antidiarrheal (dried fruits), vasodilatory, antidiabetic and vitamin agents	[18] [40] [41]
S. hybrida L.—oakleaf mountain ash (*Aria* sect. *Aria* × *Sorbus*)		An ornamental tree in northern Europe	[42]
S. pohuashanensis (Hance) Hedl. (*Sorbus*)		Fruits, stems and bark: traditional Chinese medicine for the treatment of chronic tracheitis, tuberculosis and oedema	[16]
S. sambucifolia (Cham. & Schlecht.) M. Roem.—Siberian mountain ash (*Sorbus* Lucidae Kom.)	Alcohol beverages, jams, jellies, honey (floured dried fruit)	In avitaminosis, arteriosclerosis, as antipyretic or diuretic agent.	[43]
S. scopulina Greene—Greene's mountain ash (*Sorbus*; informal group *Commixtae*)	Sometimes used in pies, preserves, or wine-making		[17]
Sorbus × *thuringiaca* (Ilse) Fritsch—mountain ash (*Aria* sect. *Aria* × *Sorbus*)		An ornamental tree	[44]
S. tianschanica Rupr. (*Sorbus* series Tianshanicae Kom.)		Leaves: asthma, ventricular myocytes, dyspnoea, tuberculosis and gastritis	[10]
S. torminalis (L.) Crantz var. *torminalis* (*Torminaria*)	Jams and ingredients for food and fodder	Traditional diuretic, anti-inflammatory, antidiarrheal (dried fruits), vasodilatory and vitamin agents	[18] [28] [40]
S. torminalis var. *semitorminalis* (*Torminaria*)		Traditional diuretic, anti-inflammatory, antidiarrheal (dried fruits), vasodilatory and vitamin agents	[18]

3. Nutritional Composition

Wild rowanberries are not consumed as fresh fruits due to their specific astringent taste, imparted mainly by the tannins. These cause the dry feeling in the mouth when consumed. Therefore, they have rather limited applications for producing food products. However, due to the nutritive value and health benefits the berries of *S. aria*, *S. aucuparia*, *S. domestica*, *S. sambucifolia*, *S. scopulina*, and *S. torminalis* have been traditionally used for pressing juice, in alcoholic beverages, purees, jams and jellies [28,35]. These benefits are due to the significant amounts of phytochemicals, such as vitamins, carotenoids, and phenolic acids as well as important in nutrition minerals, iron, potassium, and magnesium. In addition, rowanberries contain a sweet-tasting sugar alcohol sorbitol, which slowly metabolizes in the human body and therefore is suitable as a sweetener for people suffering from diabetes [45].

It was reported that rowanberries contain 3-fold higher amount of ascorbic acid than oranges [5]. For instance, Mrkonjić et al. [28] determined approximately 0.1 mg/g d.w. (dry weight) of ascorbic acid in *S. aucuparia* berries and 0.42 mg/g dw in fruit jam. The recommended dietary allowance of ascorbic acid is 60 mg per day, while 5–7 mg a day prevents scurvy. Tocopherols are important fat-soluble vitamins in rowanberries. The mean concentrations of vitamin E activity demonstrating α-tocopherol, δ-tocopherol, and γ-tocopherol in *S. aria* and *S. aucuparia* were reported 2.82, 0.11, 2.01 μg/g dw

and 4.89, 0.58, 1.71 μg/g dw, respectively [46]. Klavins et al. [47] determined even higher content of α–tocopherol (3.34 μg/g dw) in *S. aucuparia* fruit, while the content of γ-tocopherol was remarkably lower, 0.25 μg/g dw. The recommended intake of vitamin E for adults is in the range of 7 to 15 mg per day. The epidemiological studies showed that humans who consumed vitamin E richer foods had lower incidence of cancer, dementia and/or cardiovascular diseases [48].

In nature, β-carotene, a precursor (inactive form) of vitamin A, is a strongly coloured red-orange pigment, which is abundant in some plants and fruits. Berņa and Kampuse reported that *S. aucuparia* contains 2.5 mg of total carotenoids per 100 g [49]. The average daily intake of the strong antioxidant β-carotene is in the range of 2–7 mg, as estimated from a pooled analysis of 500,000 women living in the US, Canada, and some European countries [50]

The minerals are important for all living organisms. Aslantas et al. reported high content of 8 essential minerals in *S. aucuparia* (in mg/100 g): potassium, 154; phosphorus, 12.3; calcium, 29.9; magnesium, 27.84; iron, 2.42; copper, 0.294; zinc, 0.861; and manganese 0.503 [51]. The tree bark of *S. domestica* has been reported as a good source of Ca, Zn, Fe, while the seeds were rich in K, Mg, Fe and Zn [30]. Plant oils are important as food ingredients and as a source of essential fatty acids for human nutrition. In seed oils of *S. aucuparia* the sum of linoleic and oleic acids exceeded 90% of the total fatty acids [37]. Ivakhnov et al. [52] optimized the procedure for oil extraction from *S. aucuparia* alcoholic beverage production waste using the supercritical CO_2 as a solvent and recovered 9.02% (w/w) high quality oil.

4. Total Phenolic Content and Quantitative Composition of Phytochemical Antioxidants in *Sorbus* spp.

4.1. Total Phenolic Content

In general, the leaves and inflorescences of *Sorbus* spp. were reported to contain higher amounts of the total phenolic content (TPC) than the fruits (Table 2). Usually TPC is expressed in gallic acid equivalents (GAE). Thus, the highest TPC was reported in the dried leaves of *S. wilfordii* (12.31% GAE), as well as in the inflorescences of *S. aucuparia* (11.83% GAE) [53]. Predominantly, in the tested plant parts of the *Sorbus* spp., the total level of phenolics was significantly higher in the inflorescences than in the leaves [54], except for *S. gracilis*, when the TPC in the leaves was slightly higher than in the inflorescences, 11.06 and 10.72% GAE, respectively [53]. The highest TPC in fruit was detected in *S. aria* (2.98% GAE) dw; the fruits of *S. aucuparia* and *S. intermedia* contained only slightly lower TPC, 2.68% and 2.24% GAE dw, respectively [54]. The lowest TPC values among the tested *Sorbus* spp. was found in the *S. americana* fruits; it was only 3.60–5.39 mg/g dw [54,55]. Gaivelyte et al. analysed leaf and fruit material of 10 *Sorbus* spp. and 9 cultivars and found that the TPC varied approximately 5 times, both in leaf and fruit samples, i.e., in the range of 7.18–35.74 mg/g and 2.24–11.19 mg/g, respectively [56]. The berries of *S. aria* and *S. aucuparia* grown at different altitudes were compared; however, there was no correlation between TPC, total proanthocyanidins, radical scavenging capacity and growing site. Nevertheless, slightly higher TPC values were observed in *S. aucuparia*, while *S. aria* had higher content of proanthocyanidins [57].

TPC may highly depend on berry maturity, while the recovery of phenolics depends on extraction solvent. For instance, the diethyl ether fraction separated from the crude methanol extract isolated from fruit pulp of *S. domestica* berries matured at room temperature for 1 week had the highest TPC [58]. Bobinaitė et al. [59] reported the TPC in acetone, ethanol and water extracts of rowanberry pomace, which was almost similar, 10.94, 10.43 and 9.60 mg/g, respectively; while the content of individual compounds depended remarkably on the applied solvent. It was suggested that considering only slight differences in the recovery of total phenolics between the applied solvents, water would be the most attractive due to the price, availability and safety.

Olszewska et al. [37] investigated the effects of extraction with chloroform and 70% methanol and fractionation with diethyl ether, ethyl acetate, *n*-butanol of soluble in different solvents substances present in inflorescences and leaves of 7 *Sorbus* spp., namely *S. aucuparia*, *S. commixta*, *S. decora*,

S. gracilis, *S. koehneana*, *S. pogonopetala* and *S. wilfordii*. N-butanol and ethyl acetate were the most effective in recovering antioxidants from *Sorbus* leaves, whereas ethyl acetate, *n*-butanol and diethyl ether fractions of *S. pogonopetala* and *S. wilfordii* leaves contained the highest TPC, 39.56–58.17% dwe (dry weight of extract).

4.2. Phenolic Acids

Chlorogenic (3-*O*-caffeoylquinic acid, 3-CQA) and neochlorogenic acids (5-*O*-caffeoylquinic acid, 5-CQA) are the main phenolic acids reported in *Sorbus* spp. [53,55,57]. Moreover, it has been reported that caffeoylquinic acids constitute 56–80% of the total phenolics in *Sorbus* fruits, whereas the cultivated berries contain less caffeoylquinic acids than wild rowanberries [60]. The content of chlorogenic acid in the berries of *S. aucuparia* was up to 10.01 mg/g dw [57], while the content of neochlorogenic acid in the tested 5 cultivars was up to 7.31 mg/g dw [60]. Generally, the content of caffeoylquinic acids in the inflorescences was reported to be higher than in the leaves or berries. The predominant caffeoylquinic acid in the all assayed inflorescence samples was chlorogenic acid [54–57,60] with the highest concentration in *S. sambucifolia*, 4.17% dw [53] the highest contents of neochlorogenic acid were in the inflorescences of *S. koehneana* (1.98%), *S. decora* (1.26%) [53], and *S. aucuparia* (1.37%) [54]. The concentrations of chlorogenic acid in water and methanol extracts, as well as in the jam of *S. aucuparia* were 5.69, 5.80 and 2.60 mg/g dw, respectively [28]. It seems that some species instead of chlorogenic acids biosynthesize ferulic acid as the major one; in the methanol and water extracts and jams of *S. torminalis* its content was up to 62.6 μg/g dw [28].

In addition, ferulic acid content was reported in the leaves of some *Sorbus* spp., such as *S. aucuparia*, *S. aria* [43] and *S. subfusca* [61]. The methanol and water extracts and jams of both *S. torminalis* var. *torminalis* and *semitorminalis* also contained up to 23.2 μg/g dw protocatechuic acid, while in the jam of *S. aucuparia* its concentration was 12.5 μg/g dw. Protocatechuic acid was also reported in the fruits, leaves and bark of *S. alnifolia* [62], in the extracts of *S. aucuparia*, *S. commixta*, *S. gracilis*, *S. decora* and *S. koehneana* inflorescences [37], in the extracts of *S. gracilis*, *S. pogonopetala*, *S. wilfordii* [37], *S. domestica* leaves [63] and in the *S. domestica* fruit pulp [41]. Gallic acid was found only in the water extract of *S. torminalis* var. *semitorminalis* in concentration of 5.69 μg/g dw [28].

Some other well-known phenolic acids and their derivatives such as cinnamic, vanillic, *p*-coumaric and benzoic acids have been found in traces in the fruits of *S. aucuparia* [64] and *S. domestica* [41], while *p*-coumaric acid was also detected in the *S. discolor* berries [40]. Caffeic acid and its derivatives were reported in the berries of *S. aucuparia* [40], *S. domestica* [62], *S. discolor* [40], *S. alnifolia* [62], *S. pohuashanensis* [16], *S. torminalis* [40]. Vanillic acid was found in the leaves of *S. aria* [40], coumaric acid in the inflorescences of *S. aucuparia*, *S. commixta*, *S. decora*, *S. gracilis*, *S. koehneana* and in the leaves of *S. domestica* [63], *S. pogonopetala*, *S. gracilis*, and *S. wilfordii* [37].

4.3. Flavonoids

Quercetin, kaempferol, isoquercetin, rutin, hyperoside and isorhamnetin were reported in the samples of selected *Sorbus* fruits, leaves and inflorescences as the major flavonoids (Figure 2). Quercetin was the predominant flavonoid in all selected leaf and inflorescence samples and the highest values were found in the inflorescences of *S. aucuparia* (1.11% dw) followed by *S. intermedia* (1.05% dw) [18]. Among the leaf samples the highest content of quercetin was determined in *S. aucuparia* and *S. wilfordii*, 0.88% and 0.90% dw, respectively [53]. The content of quercetin in the fruits of *S. aucuparia*, *S. intermedia*, *S. aria* was 0.51, 0.31, 0.09 mg/g, respectively [54]. The highest content of isoquercetin was found in *S. commixta* fruits and leaves, 0.65 mg/g and 5.24 mg/g, respectively; among analysed rowanberries, the fruits of the same species had the highest content of hyperoside, 1.19 mg/g [56]. Kaempferol was quantified in the fruits, leaves and inflorescences of *S. aria*, *S. aucuparia* and *S. intermedia*; the highest content of this flavonoid was present in *S. aucuparia* [54]. The leaves of *S. setschwanensis* and *S. aria* were also rich in kaempferol, which constituted 0.31% [53] and 0.26% dw [54], respectively. Isorhamnetin was found only in the

fruits [40], leaves and inflorescences of *S. torminalis* [28], *S. intermedia* and *S. aria* [54]. Some isorhamnetin conjugates were also identified in *S. discolor* [40] and *S. domestica* [18]. Olszewska et al. using bioactivity-guided assay isolated several flavonoids, such as isorhamnetin 3-*O*-β-glucopyranoside, astragalin, isoquercitrin, hyperoside, kaempferol 3-*O*-β-glucopyranoside-7-*O*-α-rhamnopyranoside, quercetin 3-*O*-β-glucopyranoside-7-*O*-α-rhamnopyranoside, rutin, from the leaves of *S. aria* [65]. Among 10 investigated fruit samples of *S. aria* and *S. aucuparia* the highest content of rutin was found in the *S. aria* fruits reaching up to 892 µg/g dw [57]. Rutin was also abundant in the leaves of *S. anglica* [56].

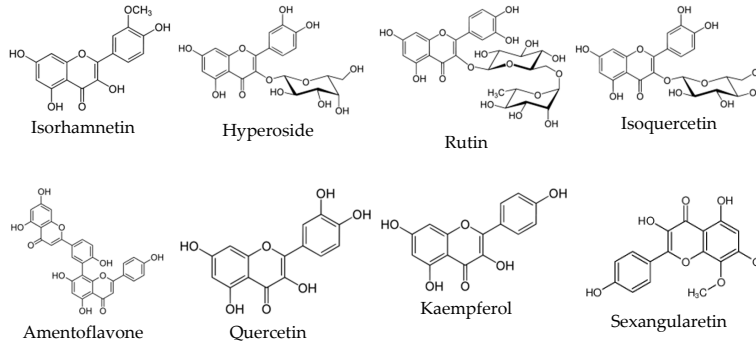


Figure 2. The structures of the main *Sorbus* flavonoids.

Table 2. Bioactive compounds in selected *Sorbus* species.

No.	Species, Tested Material and Its Isolation Method	Phenolic Acids: Total Amount (To) in GAE (%) or as Specified	Flavonoids/Proanthocyanidins in CyE (%) or as Specified	Ref.
1.	*S. americana*; 5 mL ME 10 mL ME 20 mL ME of F	All in mg/g dwe: To 3.599; 5-CQA 0.662; 3-CQA 2.837; QG 0.101 To 4.432; 5-CQA 0.714; 3-CQA 3.599; QG 0.119 To 5.388; 5-CQA 0.905; 3-CQA 0.417; QG 0.066		[55]
	S. americana; 70% ME of L	To 6.47; 5-CQA 0.04; 3-CQA 1.85	Fl: QU 0.46; KA 0.04; PAC 3.66	[53]
2.	*S. aria*; 70% ME of I, F & L	I: To 6.58; 5-CQA 1.18; 3-CQA 1.78 L: To 6.06; 5-CQA 0.99; 3-CQA 0.74 F: To 2.98; 5-CQA 0.32; 3-CQA 0.30	Fl in I: QU 0.277; SX 0.050; KA 0.041; IS 0.284 Fl in L: QU 0.493; SX 0.014; KA 0.242; IS 0.095 Fl in F: QU 0.009; KA 0.002; IS 0.007 PAC: I 2.75; L 3.53; F 1.80	[54]
	S. aria; ME of I, F & L		Fl, mg/100 g; F: Ag 20.3; Gl 31.1; QU 9.4; KA 2.4; IS 8.5 I: Ag 687.2; Gl 1049.0; QU 291.6; SX 52.6; KA 43.7; IS–299.3 L: Ag 888.1; Gl 1371.9; IS 99.8; QU 518.9; SX 14.8; KA 254.6	[18]
	S. aria; EtE of F	In mg/g dw; To: 3.91–10.81; 5-CQA: 0.18–4.00; 3-CQA: 0.22–2.30	Fl, µg/g dw; RU: 138.4–892.0; HY: 2.3–27.6; IQ: 10.9–108.6; QU: 2.1–35.2 PAC, mg/g dw: avr. 1.11	[57]
3.	*S. aucuparia*; 70% ME of I	To 21.17; DEF 37.61; EtAF 54.34; BF 48.71; WR 9.05		[43]
	S. aucuparia; AE of F	To 190 mg/100 g dw	Fl-To 68.1 mg/100 g dw	[49]
	S. aucuparia; 80% AE of F	To 0.2148; 5-CQA 0.0427; 3-CQA 0.0705	PAC 0.0005	[66]
	S. aucuparia; 70% AE of F	mg/g dw; wild F: 5-CQA 5.36; 3-CQA 8.59; other 1.84; HB 0.11. Cultivars: 5-CQA 2.23–7.31; 3-CQA 3.20–9.22; other 0.61–1.84; HB: 0.16–0.70	mg/g dw; wild: flavonols 1.84; flavanols 0.97; PAC 0.12. Cultivars: Fl 0.94–1.88; flavonols 0.95–1.89; PAC 0.36–6.04	[60]
	S. aucuparia; ME of F	Cultivars 4.35–8.19	Fl, g/kg fm: wild 3.11	[34]
	S. aucuparia; WE of F, ME of F & jam	µg/g dwe: WE-F: 3-CQA 5.69x10^3; FA 7.8 ME-F: 3-CQA 5.80x10^3; FA 9.59 Jam: PCA 12.5; 3-CQA 2.60x10^3; FA 11.4	Fl, µg/ g dwe; WE-F: AF 10.7; KA-3-*O*-gl 9.0; QU-3-*O*-gl 49.3; HY 36.6; RU 82.3 ME-F: AF 11.9; KA-3-*O*-gl 8.56; QU-3-*O*-gl 55.8; HY 39.6; RU 80.4 Jam: AF 8.4; KA-3-*O*-gl 3.99; QU-3-*O*-gl 17.9; HY 9.68	[28]

Table 2. *Cont.*

No.	Species, Tested Material and Its Isolation Method	Phenolic Acids: Total Amount (To) in GAE (%) or as Specified	Flavonoids/Proanthocyanidins in CyE (%) or as Specified	Ref.
	S. aucuparia; 70% AE of cultivars	In mg/ 100 g fw: To 550–1014; 5-CQA 34–104; 3-CQA 29–160	PAC 6–80 mg/100 g fw	[31]
	S. aucuparia; 70% ME of I, F & L	I: To 11.83; 5-CQA 1.37; 3-CQA 2.98 L: To 9.09; 5-CQA 1.15; 3-CQA 2.75 F: To 2.68; 5-CQA 0.29; 3-CQA 0.64	I: (Fl) QU 1.054; SX 0.151; KA 0.071. PAC 5.01 L: (Fl) QU 0.835; KA 0.188. PAC 3.84 F: (Fl) QU 0.051; KA 0.006. PAC 1.07	[54]
	S. aucuparia EtE of F	In mg/g dw: To 5.25–15.91; 5-CQ 0.67–7.03; 3-CQA 0.3510.01	Fl, µg/g dw: RU 40.1–598.3; HY 2.4–559.9; IQ 6.1–252.8; QU 2.8–83.5. PAC (avr.) 0.92 mg/g dw	[57]
	S. aucuparia; ME of I, F & L		Fl, mg/100 g: (L) Ag 1078; Gl 1666; QU 881.1; KA 196.9. (F) Ag 60.2; Gl 92.9; QU 53.8; KA 6.4. (I) Ag 1344.1; Gl 2067.4; QU 1110.7; SX 0.1582; KA 75.2	[18]
	S. aucuparia; 70% ME of I & L	I: To 10.02; 5-CQA 0.74; 3-CQA 2.27 L: To 8.23; 5-CQA 0.51; 3-CQA 1.90	Fl: (I) QU 1.048; SX 0.190; KA 0.084 (L) QU 0.903; KA 0.157. PAC: I 5.94; L 3.59	[53]
4.	*S. caslmiriana;* 70% ME of L	L: To 5.78; 5-CQA 0.37; 3-CQA 1.25	Fl: QU 0.532; KA 0.113. PAC 4.02	[53]
5.	*S. tianschanica;* 50% EtE of F& L	F, mg/g: 5-CQA 3.7; 3-CQA 2.6. L.: 5-CQA 6.0; 3-CQA 7.0	Fl, mg/g: (F) RU 0.15; HY 0.08; IQ 0.32. (L) RU 1.5; HY 1.4; IQ 5.1	[56]
	S. tianschanica; WE of L		Fl, mg/g: RU 0.71; HY 1.18; HE 0.48	[67]
6.	*S. commixta;* 50% EtE of L & F	L, mg/g: To 35.74; 5-CQ-.1.10; 3-CQA-21.91. F: To-11.19; 5-CQA- 1.8; 3-CQA-7.5	Fl, mg/g: L: HY-7.5; IQ-5.3. F: HY-1.20; IQ-0.65; RU-0.02	[56]
	S. commixta; hot-WE and 70% EtE of S	To in µg/mg: We 364.64; EtE 504.39	To-Fl, µg/mg: WE 124.59; EtE 160.09	[68]
	S. commixta; 70% EtE of C	To, µg/mg: Without enzyme 447.3; treated with: amylase 501.6; amyloglucosidase 461.2; glucosidase 510.7; glucanase 493.3; cellulase 449.6	Fl, µg/mg: without enzyme 35.1; treated with: amylase 55.1; amyloglucosidase 41.4; glucosidase 51.3; glucanase 63.0; cellulase 36.8	[69]
	S. commixta; 70% ME of I and fractions (f)	ME 21.17; DEf 37.61; EtAf 54.34; Buf 48.71; WR 9.05		[37]
	S. commixta; 70% ME of I & L	I: To 9.29; 5-CQ 0.76; 3-CQA 3.92 L: To 8.08; 5-CQ 0.05; 3-CQA 0.79	Fl: (I) QU 0.422; KA 0.050; SX 0.045 (L) QU 0.470; KA 0.011. PAC: I 5.98; L 3.58	[53]
7.	*S. decora;* 70% ME of I & L	I: To 11.67; 5-CQA 1.26; 3-CQA 3.85 L: To 8.10; 5-CQA 0.19; 3-CQA 2.10	Fl: (I) QU 0.839; KA 0.059; SX 0.07. (L) QU 0.474; KA 0.035. PAC: I 6.40; L 4.03	[53]
	S. decora; 70% ME of I	ME 24.61; DEf 34.50; EtAf 55.16; Buf 53.75; WR 10.06		[37]
8.	*S. domestica;* ME of (1), (2), (3), (4), (5)	To, µg/mg: R: 13.6 (1) → 25.4 (2) → 20.5 (3) → 32.1 (4) → 30.2 (5); DCMF: 74.5 (1) → 27.0 (2) → 97.0 (3) → 66.5 (4); DEf: 245(1) →151(2) → 324 (3) → 148(4) → 143 (5); EtAf: 285 (1) → 137 (2) → 198 (3) → 64(4) → 341(5); Buf: 94.0 (1) → 16.1 (2) → 25.1 (3) → 12.5 (4) →140 (5); Wf: 14.8 (1) → 3.03 (2) → 11.3 (3) → 2.27 (4) → 34.4 (5); ME: 32.5 (1) → 10.3 (2) → 26.3 (3) → 5.58 (4) → 28.1 (5)		[58]
	S. domestica; ME of (1), (2), (3), (4), (5)	(1): To 14.72; CiA 10.55; BA 4.17. (2): To 18.85; CiA 9.91; BA 8.94. (3): To 18.18; CiA 14.24; BA 4.57. (4): To 19.28; CiA 12.19; BA 7.09. (5): To 4.86; CiA 2.55; BA 2.31	Fl: (1) To 8.68; Ag 1.22; Gl 7.46. (2) To 3.08; Ag 0.36; Gl 2.72. (3) To 10.59; Ag 1.46; Gl 8.83. (4): To 2.45; Ag 0.46; Gl 1.99. (5) To 7.9; Ag 0.73; Gl 7.17	[41]
9.	*S. gracilis;* 70% ME of I & L	I: To 11.06; 5-CQA 0.19; 3-CQA 3.31 L: To 10.72; 5-CQA 0.03; 3-CQA 0.93	Fl: (I) QU 0.194; KA 0.012; SX 0.072 (L) QU 0.113; KA 0.008. PAC: I 6.54; L 6.56	[53]
	S. gracilis; 70% ME of I & L	I: ME 24.63; DEf-36.87; EtAf 54.09; Buf 57.09; WR 8.21. L: ME 30.62; DEf 34.90; EtAf 52.37; Buf 48.62; WR 11.45		[37]
10.	*S. intermedia;* ME of I, F & L		Fl, mg/100g: (I) Ag 1514.8; Gl 2320.7; QU 1053.4; SX 117.3; KA 29.3; IS 314.8. (L) Ag 424.1; Gl 652.6; QU 303.6; KA 52.0; IS 68.5 (F) Ag 44.4; Gl 68.2; QU 32.5; IS 9.5; KA 2.4	[18]
	S. intermedia 70% ME of I, F & L	I: To 9.25; 5-CQA 0.68; 3-CQA 2.35 L: To 8.74; 5-CQA 0.65; 3-CQA 1.26 F: To 2.24; 5-CQA 0.27; 3-CQA 0.23	Fl: (I) QU 0.277; SX 0.05; KA 0.041; IS 0.284. PAC 5.52 (L) QU 0.493; SX 0.014; KA 0.242; IS 0.095. PAC 5.45 (F) QU 0.009, KA 0.002; IS 0.007. PAC 0.82	[54]

Table 2. *Cont.*

No.	Species, Tested Material and Its Isolation Method	Phenolic Acids: Total Amount (To) in GAE (%) or as Specified	Flavonoids/Proanthocyanidins in CyE (%) or as Specified	Ref.
11.	*S. koehneana;* ME of I & L	I: To 11.67; 5-CQA 1.98; 3-CQA 2.05 L: To 9.87; 5 CQA-0.53; 3-CQA 1.97	Fl: (I) QU 0.27; KA 0.02; SX 0.05. PAC 6.86 L: QU 0.25; KA 0.11. PAC 5.81	[53]
	S. koehneana; 70% ME of I & L	To: ME 26.38; DEf32.10; EtAf50.51; Buf 58.17; WR 10.51		[37]
12.	*S. pohuashanensis;* 70% ME of I & L	I: To 11.32; 5-CQA 0.7; 3-CQA 2.48 L: To 6.26; 5-CQA 0.12; 3-CQA 0.67	Fl: I: QU-0.4; KA-0.04; SX-0.02. L: QU-0.12; KA-0.03. PAC: I-7.67; L-3.93	[53]
13.	*S. pogonopetala;* 70% ME of L	To 10.9; 5-CQA 0.22; 3-CQA 1.63	Fl: QU 0.38; KA 0.26. PAC 5.89	[53]
	S. pogonopetala; 70% ME of L	To: ME 24.03; Def 42.85; EtAf 53.29. Buf 39.56; WR 10.38		[37]
14.	*S. sambucifolia;* 70% ME of I & L	I: To 8.2; 5-CQA 0.42; 3-CQA 4.17. L: To 5.07; 5-CQA 0.1; 3-CQA 1.02	Fl: (I) QU 0.81; KA 0.06; SX 0.13. PAC 3.79 (L) QU 0.16; KA 0.01. PAC 1.96	[53]
	S. sambucifolia; EtE of F	To 0.733	Fl: To 0.002	[70]
15.	*S. scalaris;* 70% ME of I & L	I: To 8.47; 5-CQA 0.6; 3-CQA 2.36 L: To 4.23; 5-CQA 0.36; 3-CQA 1.24	Fl: (I) QU 0.34; KA 0.06; SX 0.15. PAC 5.68 (L) QU 0.22; KA 0.13. PAC 1.47	[53]
16.	*S. setschwanensis;* 70% ME of L	To 10.18; 5-CQA 0.22; 3-CQA 2.61	Fl: QU 0.57; KA 0.31. PAC 5.56	[53]
17.	*S. sitchensis;* 70% ME of I & L	I: To 10.08; 5-CQA 0.45; 3-CQA 3.13 L: To-4.89; 5-CQA- 0.05; 3-CQA-0.56	Fl: (I) QU 0.38; KA 0.02; SX 0.05 L: QU-0.27; KA-0.02. PAC: I-7.14; L-1.48	[53]
18.	*S. torminalis* var. *torminalis;* WE of F, ME of F & jam	In µg/g dwe; WE-F: PCA 13.7; FA 27.8 ME-F: PCA 23.2; FA 62.6 Jam: PCA 5.92; FA 13.3	Fl, µg/g dwe; WE-F: AF 15.8 ME-F: AF 19.3; QU-3-*O*-gl 13.6; HY 10.4 Jam: AF 16.8; QU-3-*O*-gl 2.53; HY 1.61	[28]
	S. torminalis var. *semitorminalis;* WE of F, ME of F & jam	WE-F: GA 5.69; FA 43.3; PCA 4.61 ME-F: FA 38.3; PCA 3.44 Jam: FA 18.4; PCA 2.11	Fl, µg/g dwe; WE-F: AF 362; KA-3-*O*-gl 2.34; QU 6.53; QU-3-*O*-gl 3.33; Cat 10.6 ME-F: AF 974; KA-3-*O*-gl 2.43; QU 11; QU-3-*O*-gl 2.06 Jam: AF 195; QU 3.76; QU-3-*O*-gl 1.60	[28]
19.	*S. wilfordii;* 70% ME of L	To 12.31; 5-CQA 0.13; 3-CQA 2.58 ME 29.93; DEf 53.13; EtAf 54.34; Buf 48.37; WR 15.27	Fl: QU-0.88; KA-0.05. PAC: 5.31	[53]
	S. wilfordii; 70% ME of L			[37]

F—fruits; L—leaves; I—inflorescences; S—stems; C—cortex, B—bark. M—methanol; Et—ethanol; A—acetone; DCM—dichloromethane; DE—diethyl ether; Bu—butanol; EtA—ethyl acetate; W—water; E—extract; R—residue; f—fraction. Total phenolic content is expressed in GAE (gallic acid equivalents); avr—average; fm—fresh mass; Fl—flavonoids in %; PAC—proanthocyanidins in % of CyE (cyanidin chloride equivalents); 3-CQA—chlorogenic acid; 5-CQA—neochlorogenic acid; GA—gallic acid; HC—hydroxycinnamic acid; CA—caffeic acid; p-c—p-coumaric; HB—hydroxybenzoic; Gl—glycoside, Ag—aglycone, PCA—protocatechuic acid; CiA—cinnamic acids, BA—benzoic acids; FA—ferulic acid; AF—amentoflavone; QG—quercetin-3-*O*-glucoside; KA-3-*O*-gl—kaempferol-3-*O*-glucoside; Cat—catechin; QU-3-*O*-so—quercetin-3-*O*-β-sophoroside; QU—quercetin; KA—kaempferol; SX—sexangularetin. Unripe fruit (1), well matured on tree (2), matured for 1 week at room temperature (3), matured for 3 weeks at room temperature (4), fruit pulp from well matured fruits (5).

Sexangularetin was one of the most abundant flavonoid component in the inflorescences; *S. aucuparia* and *S. scalaris* contained 0.19% and 0.14% dw, respectively [53]. Epicatechin was reported in the leaves of many *Sorbus* spp. [71,72], as well as in the berries of *S. aucuparia* [60] and *S. torminalis* var. *semitorminalis* [28]. Hesperidin was found only in the leaves of *S. tianschanica* [67,73]. The highest levels of proanthocyanidins among the inflorescences of 12 tested species were found in the *S. pohuashanensis* and *S. sitchensis*, 7.67% and 7.14% CyE, respectively. Among the 17 leaf samples, *S. gracilis* had the highest concentration of proanthocyanidins, 6.56% CyE [53]. Among the rowanberries, the highest content of proanthocyanidins was found in the fruits of *S. aria*, 1.80% CyE [54]. Catechin and epicatechin were the main flavonoid components in the samples of *S. decora* stembark [74], rootsock [75], but also in the water extract of *S. torminalis* var. *semitorminalis* [28].

Quercetin content in methanol extract of *S. torminalis* var. *semitorminalis* was 11.0 µg/g while in water extract it was 2-fold lower, 6.53 µg/g [28]. The content of rutin in water and methanol extracts of *S. aucuparia* was found similar, 82.3 and 80.4 µg/g dw, respectively [28]. Hydroethanolic (70%) extract of dried *S. commixta* stems and cortex contained higher by 50.43% total polyphenol and flavonoid content than water extract; the former also demonstrated stronger antioxidant capacity [68].

Exceptionally high content of amentoflavone was found in *S. torminalis* var. *semitorminalis* water and methanol extracts as well as in its jam, namely 362, 974, and 195 μg/g dw, respectively. However, its content in the extracts and jam of *S. torminalis* var. *torminalis* and *S. aucuparia* differed just slightly: it was 15.8, 19.3 and 16.8 μg/g dw and 10.7, 11.9 and 8.4 μg/g dw, respectively [28]. Up to 119 μg/g dw of quercetin-3-*O*-glucoside were reported in *S. americana* [55]. Typically, anthocyanins have been detected in the *S. aucuparia* cultivars however only in low concentrations, usually less than 1% of the total phenolics in the wild fruits [60]. Bobinaitė et al. [59] reported that the total content of proanthocyanidins in *S. aucuparia* pomace water extract was 10.4 and 3.8 times, higher than that in the acetone and ethanol extracts, respectively.

5. Antioxidant Potential of *Sorbus* spp.

Plant material, suitable for cost-effective production of natural antioxidants should contain reasonable amount of polyphenolics (usually not less than 8–10% GAE/dw), demonstrate comparatively strong antioxidant properties in several assay systems and exhibit as low as possible toxicity, which should be acceptable for human applications [53].

Large number of phytochemicals belonging to various classes of organic compounds have been identified in various *Sorbus* spp. [4]. The presence of significant amounts of polyphenolic antioxidants, mainly flavonoids and phenolic acids, has also been reported in *Sorbus* spp. (Table 2). Moreover, many authors observed good positive correlation between the concentration of phenolics, e.g., the sum of proanthocyanidins, caffeoylquinic acids and flavonoid aglycones and antioxidant properties [31,57,58]. Therefore, in many studies rowanberries exhibited significant antioxidant activity (Table 3), which was comparable or in some cases even higher than that of many other edible berries, such as chokeberries and bilberries [76]. Various methods have been applied for assessing antioxidant properties of rowanberries and their extracts, most frequently using the in vitro radical scavenging capacity assays and inhibition of lipid peroxidation [77], reducing power, chain-breaking potential of radical reactions [60] and others. The majority of studies investigated *Sorbus* fruits, leaves and inflorescences; however, antioxidant properties of tree bark and seed oil were also reported [46].

The main polyphenolic compounds responsible for antioxidant properties of rowanberries are phenolic acids (mostly caffeoylquinic acids), flavonols (quercetin, isoquercetin, hyperoside, rutin, catechin, epicatechin), anthocyanins (mainly cyanidin or pelargonidin glycosides), and proanthocyanidins [53,60]. In addition, many studies have reported several quercetin, sexangularetin (SX) and kaempferol (KA) glycosides in the fruits, inflorescences, leaves and stems of various *Sorbus* spp. (Table 2).

The stage of maturity [45], genotype [40], species [53], geographic origin [44], climatic environment, as well as storage conditions [78] and treatment [28] affect the composition of bioactive constituents. For example, Mrkonjić et al. [28] reported that among 12 identified in *S. aucuparia* and *S. torminalis* phenolic compounds chlorogenic acid was the most abundant in the former, while flavonoid amentoflavone in the latter one. The fruits of *S. aucuparia* better scavenged DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl), $^{\bullet}$NO, $O_2^{\bullet}$, HO$^{\bullet}$ and inhibited lipid peroxidation (LP) than those of *S. torminalis*; however, both varieties of the latter species, namely *torminalis* and *semitorminalis* demonstrated almost identical antioxidant potential.

Many researchers have reported the correlation between the TPC and antioxidant capacity, particularly in case of using very popular chemical in vitro assays such as DPPH$^{\bullet}$/ABTS$^{\bullet+}$ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) scavenging, FRAP (ferric reducing antioxidant power) and LPO (inhibition of lipid peroxidation). The ethyl acetate (EtOAc) extract of *S. americana* berries and other nine edible North American plants were tested for antioxidant activity using the DPPH$^{\bullet}$ scavenging assay. DPPH$^{\bullet}$ scavenging value IC_{50} of *S. americana* was 113.96 μg/mL; other in this study investigated plants, *Gaultheria shallon* and *Sambucus cerulea* exhibited stronger antioxidant capacity with IC_{50} values of 14.76 and 29.32 μg/mL, respectively [36]. Methanol extracts of *S. americana* dried bark and leaves were remarkably stronger DPPH$^{\bullet}$ scavengers with EC_{50} of 15.80 μg/mL [12] and

38.76 µg/mL [53], respectively. For comparison, these values for black tea and coffee were 15.19 and 40.32 µg/mL, respectively [77].

Hukkanen et al. reported high antioxidant activity and phenolic contents in the fruits of several sweet rowanberry (*S. aucuparia*) cultivars, namely Burka, Dessertnaja, Eliit, Granatnaja, Kubovaja, Rosina, Rubinovaja, Titan, and Zholtaja: DPPH$^{\bullet}$ scavenging capacity and FRAP values were in the ranges of 9.7–21.3 g fw/g radical and 61–105 mmol Fe(II)/g fw [31]. Olszewska et al. analysed different anatomical parts of *S. aucuparia*, *S. aria* and *S. intermedia* and found that *S. aucuparia* inflorescence demonstrated the highest antioxidant capacity: in FRAP and ABTS$^{\bullet+}$ decolouration assays it was 2453.5 µmol TE/g dw and 83.05 mg/L, respectively, while the IC_{50} in DPPH$^{\bullet}$ scavenging assay was 18.05 µg/mL. Respective values of *S. aria* fruit were 497.7 µmol TE/g dw (FRAP), 142.20 mg/L (ABTS$^{\bullet+}$) and 95.31 µg/mL (DPPH$^{\bullet}$) [54].

The same authors measured DPPH$^{\bullet}$ scavenging of 16 *Sorbus* spp. and determined that the lowest EC_{50} values demonstrated methanolic extracts of *S. aucuparia*, *S. pohuashanensis*, *S. decora*, *S. koehneana*, *S. commixta*, *S. gracilis*, and *S. sitchensis* inflorescences and *S. wilfordinci*, *S. pogonopetala*, *and S. gracilis* leaves; they were in the ranges of 16.20–27.21 µg/mL and 15.23–20.71 µg/mL, respectively. These results correlated with high total phenolic levels [53]. Mrkonjić et al. [28] observed that methanolic and water extracts and jams of *S. aucuparia* fruits were stronger antioxidants than *S. torminalis.*

Table 3. Bioactivity of selected *Sorbus* species.

No.	Species, Tested Material and Its Isolation Method	Antioxidant Activity EC_{50} (µg/mL) or as Specified	TEAA, mmol/g or LPO%	FRAP, mmol Fe^{2+}/g or as Specified	Ref.
1.	*S. alnifolia*; 75% EtE of L	DPPH$^{\bullet}$ 30.6			[39]
2.	*S. americana*; 70% ME of L	DPPH$^{\bullet}$ 38.76	TEAA-0.34; LPO-54.29		[53]
	S. americana; EtAE of F	DPPH$^{\bullet}$ 113.9			[12]
	S. americana ME of B	DPPH$^{\bullet}$ 15.8			[36]
3.	*S. aria*; 70% ME of I, L & F	DPPH$^{\bullet}$: I 42.05; L 50.17; F 95.31	TEAA: I 0.41; L 0.344; F 0.18	I 1.394; L 1.119; F 0.498	[54]
	S. aria EtE of F	DPPH$^{\bullet}$, mg/mL: 0.49−2.50			[57]
4.	*S. aucuparia*; 70% ME	DPPH$^{\bullet}$: ME 8.93; DEf 5.53; EtAf 3.37; Buf 3.52; WR 9.96	TEAA: ME 1.72; DEf 2.14; EtAf 3.22; Buf 3.58; WR 0.94	ME 4.43; DEf 9.30; EtAf 12.77; Buf 10.84; WR 2.58	[37]
	S. aucuparia; ME of F and cultivars	ME-F, DPPH$^{\bullet}$, g/kg fm: 6.73; % of inhibition: HO$^{\bullet}$ 16.33; $O_2^{\bullet}$ 26.74; $^{\bullet}$NO 24.75. Cultivars: DPPH$^{\bullet}$ 6.58−9.62; % of inhib.: HO$^{\bullet}$ 16.12–24.73; $O_2^{\bullet}$ 27.19–34.02; $^{\bullet}$NO 25.03–31.39	ME-F LPO, % of inhibition: 8.21 Cultivars: 7.93–13.12		[34]
	S. aucuparia; AE of F	DPPH$^{\bullet}$, mmol/kg dw: 357		mmol Fe^{2+}/ kg dw: 315.5	[49]
	S. aucuparia; WE of F, ME of F & jam	WE: DPPH$^{\bullet}$ 70; $^{\bullet}$NO 1430; $O_2^{\bullet}$ 20.16 × 10^6; HO$^{\bullet}$ 160 ME: DPPH$^{\bullet}$ 80; $^{\bullet}$NO 430; $O_2^{\bullet}$ 20.5 × 10^6; HO$^{\bullet}$ 240 Jam: DPPH$^{\bullet}$ 130; $^{\bullet}$NO 2260; $O_2^{\bullet}$ 67.8 × 10^6; HO$^{\bullet}$ 610	LPO mg mL: WE-F - 6.40; ME-F - 7.38; jam - 4.08	mg of AAE/g: WE-F: 10.6. ME-F: 11.2. Jam: 4.22	[28]
	S. aucuparia (sweet cultivars); 70% AE	DPPH$^{\bullet}$, g/g: 21.3–9.7		0.061−0.105	[31]
	S. aucuparia; 70% ME of I, L, & F	DPPH$^{\bullet}$: I 18.05; L 27.47; F 163.63	TEAA: I 0.956; L 0.628; F 0.106	I 2.454; L 2.148; F 0.442	[54]
	S. aucuparia; 70% ME of I & L	DPPH$^{\bullet}$: I 16.69; L 24.10	TEAA: I 0.78; L 0.54 LPO: I 68.34; L 58.69		[53]
	S. aucuparia EtE of F	DPPH$^{\bullet}$ 340−4260			[57]
5.	*S. cashmiriana*	In µmol/mL; DPPH$^{\bullet}$ 7.6−12.5; H_2O_2 15.4−18.6; ABTS$^{\bullet+}$ 18.3−24.4		µmol/mL:11.3−23.8	[15]
	S. cashmiriana; 70% ME of L	DPPH$^{\bullet}$ 48.59	TEAA 0.27; LPO 53.59		[53]
6.	*S. commixta*; hot-WE of S	In % of inhibition: (50 µg/L) $^{\bullet}$OH 10.37; $^{\bullet}$NO 92.63 (pH1.2), 66.82 (pH3). (25 µg/L) $^{\bullet}$OH 10.08; $^{\bullet}$NO 65.36 (pH1.2); 41.06 (pH3). (12.5 µg/L) $^{\bullet}$OH 7.63; $^{\bullet}$NO 42.59(pH1.2), 26.78 (pH3). (10 µg/L): DPPH$^{\bullet}$ 21.39; ABTS$^{\bullet+}$ 43.21. (5 µg/L) DPPH$^{\bullet}$ 12.75; ABTS$^{\bullet+}$ 24.96. (1 µg/L) DPPH$^{\bullet}$ 5.27; ABTS$^{\bullet+}$ 8.77		In %: 50 µg/L 19.28; 25 µg/L 9.28 12.5 µg/L 6.83	[68]
	S. commixta; 70% EtE of S	In % of inhibition: (50 µg/L) $^{\bullet}$OH 23.61; $^{\bullet}$NO 96.64 (pH1.2), 82.51 (pH3). (25 µg/L) $^{\bullet}$OH 22.15; $^{\bullet}$NO 91.97 (pH1.2), 80.02 (pH3). (12.5 µg/L) $^{\bullet}$OH 18.42; $^{\bullet}$NO 86.55 (pH1.2), 72.44 (pH3). (10 µg/L) DPPH$^{\bullet}$ 26.36; ABTS$^{\bullet+}$ 59.64. (5 µg/L) DPPH$^{\bullet}$ 15.96; ABTS$^{\bullet+}$ 37.01. (1 µg/L) DPPH$^{\bullet}$ 6.93; ABTS$^{\bullet+}$ 12.14.		In %: 50 µg/L 13.06 25 µg/L 10.31; 12.5 µg/L 9.30	[68]

Table 3. *Cont.*

No.	Species, Tested Material and Its Isolation Method	Antioxidant Activity EC_{50} (μg/mL) or as Specified	TEAA, mmol/g or LPO%	FRAP, mmol Fe^{2+}/g or as Specified	Ref.
	S. commixta; 70% EtE of C	Without enzyme: $O_2^{\bullet}$ 14.2; DPPH$^{\bullet}$ 18.0. Amylase: $O_2^{\bullet}$ 14.8; DPPH$^{\bullet}$ 15.4. Amyloglucosidase: $O_2^{\bullet}$ 14.2, DPPH$^{\bullet}$ 15.8. Glucosidase: $O_2^{\bullet}$ 13.8, DPPH$^{\bullet}$ 15.7. Glucanase: $O_2^{\bullet}$ 13.6, DPPH$^{\bullet}$ 15.2. Cellulase: $O_2^{\bullet}$ 14.6, DPPH$^{\bullet}$ 18.2			[69]
	S. commixta; 70% ME, f and R	DPPH$^{\bullet}$: ME 7.16; DEf 5.72; EtAf 3.52; Buf 3.53; WR 9.66	TEAA: ME 1.70; DEf 2.14; EtAf 2.62; Buf-3.40; WR 1.26	ME 5.04; DEf 7.58; EtAf 12.23; Buf 11.01; WR 2.70	[37]
	S. commixta; 70% ME of I & L	DPPH$^{\bullet}$: I 23.22; L 28.56	TEAA: I 0.56, L 0.46 LPO: I 78.21, L 58.65		[53]
7.	*S. decora*; 70% ME, Fs and R of I	DPPH$^{\bullet}$: ME 7.76; DEf 5.57; EtAf 3.44; Buf 3.17; WR 9.84	TEAA: ME 1.79; DEf 2.67; EtAf 3.98; Buf 3.55; WR 1.21	ME 5.42; DEf 8.5; EtAf 13.74; Buf 11.47; WR 2.77	[37]
	S. decora; 70% ME of I & L	DPPH$^{\bullet}$: I 16.20; L 27.21	TEAA: I 0.81; L 0.48 LPO: I 70.99; L 59.99		[53]
8.	*S. domestica*; ME of (1), (2), (3), (4), (5)	DPPH$^{\bullet}$: (R) 4829(1)→6290(2)→3720(3)→2730(4)→1810(5). DCMf: 3600(1)→9880(2)→3820(3)→6010(4). DEf: 997(1)→1740(2)→825(3)→3280(4)→ 2970(5). (EtAf) 1780(1)→1750(2)→1840(3)→3170 (4)→899(5). (Buf) 588(1)→8000(2)→3750 (3)→13200(4)→341(5). Wf: 4950(1)→ 39100(2)→5570(3)→39500(4)→2170(5). (ME) 2550(1)→10600(2)→1890(3)→20000(4)→1450(5)			[58]
9.	*S. gracilis*; 70% ME of I & L	DPPH$^{\bullet}$: (I) ME 7.93; DEf 5.39; EtAf 3.71; Buf 3.25; WR 10.12. (L) ME 6.60; DEf 5.29; EtAf 3.70; Buf 3.83; WR 9.54	TEAA: (I) ME 1.99; DEf 2.71; EtAf 3.65; Buf 3.68; WR 1.15. (L) ME 2.12; DEf 2.14; EtAf 3.72; Buf 3.33; WR 1.31	I: ME 5.36; DEf 9.34; EtAf 13.06; Buf-9.92; WR 2.26. (L) ME 6.2; DEf 8.72; EtAf 12.94; Buf 11.05; WR 2.98	[37]
	S. gracilis; 70% ME of I & L	DPPH$^{\bullet}$: I 19.09; L 20.71	TEAA: I 0.68; L 0.63 LPO: I 73.01; L 70.72		[53]
10.	*S. intermedia*; 70% ME of I, L & F	DPPH$^{\bullet}$: I 25.41; L 30.71; F 198.69	TEAA: I 0.679; L 0.572; F 0.087	I 2.123; L 1.512; F 0.298	[54]
11.	*S. koehneana*; 70% ME of I & L	DPPH$^{\bullet}$: I 16.20; L 24.74	TEAA: I 0.81; L 0.53 LPO: I 73.34; L 54.15		[53]
	S. koehneana; 70% ME of I	DPPH$^{\bullet}$: ME 6.74; DEf 5.70; EtAf 3.46; Buf 3.15; WR 9.71	TEAA: ME 2.08; DEf 2.60; EtAf 3.56; Buf 3.94; WR 1.29	ME 5.44; DEf 8.38; EtAf 12.87; Buf 9.81; WR 2.54	[37]
12.	*S. pohuashanensis*; 70% ME of I & L	DPPH$^{\bullet}$: I 17.89; L 43.86	TEAA: I 0.73; L 0.30 LPO: I 68.69; L 50.21		[53]
13.	*S. pogonopetala*; 70% ME of L	DPPH$^{\bullet}$: ME 6.84; DEf 4.89; EtAf 3.8; Buf 5.18; WR 9.83	TEAA: ME 1.81; DEf 2.28; EtAf 3.44; Buf 2.96; WR 1.03	ME 5.54; DEf 10.92; EtAf 11.42; Buf 8.67; WR 2.92	[37]
	S. pogonopetala; 70% ME of L	DPPH$^{\bullet}$ 19.87	TEAA 0.66; LPO 74.73		[53]
14.	*S. sambucifolia*; 70% ME of I & L	DPPH$^{\bullet}$: I 28.03; L 52.63	TEAA: I 0.47; L 0.25 LPO: I 58.12; L 54.03		[53]
15.	*S. scalaris*; 70% ME of I & L	DPPH$^{\bullet}$: I 27.65; L 57.86	TEAA: I 0.47; L 0.23 LPO: I 55.23; L 41.70		[53]
16.	*S. setschwanensis*; 70% ME L	DPPH$^{\bullet}$ 23.30	TEAA 0.56; LPO 63.77		[53]
17.	*S. sitchensis*; 70% ME of I & L	DPPH$^{\bullet}$: I 20.75; L 54.23	TEAA: I 0.63; L 0.24 LPO: I 68.26; L 53.13		[53]
18.	*S. torminalis* (L.) Crantz var. *torminalis*; WE of F, ME of F & jam	WE-F: DPPH$^{\bullet}$ 1380; $O_2^{\bullet}$ 7.09×10^6; HO$^{\bullet}$ 300. ME-F: DPPH$^{\bullet}$ 570; $O_2^{\bullet}$ 12.2×10^6, $^{\bullet}$NO 2820; HO$^{\bullet}$ 260. Jam: DPPH$^{\bullet}$ 440; $^{\bullet}$NO 640; $O_2^{\bullet}$ 36.9×10^6; HO$^{\bullet}$ 1110		mg AAE/g: WE-F 1.11; ME-F 2.12; jam: 3.1	[28]
19.	*S. torminalis* var. *semitorminalis*; WE of F, ME of F & jam	WE-F: DPPH$^{\bullet}$ 1270; $O_2^{\bullet}$ 12.8×10^6; HO$^{\bullet}$ 430. ME-F: DPPH$^{\bullet}$ 420; $^{\bullet}$NO 3.12; $O_2^{\bullet}$ 12.5×10^6; HO$^{\bullet}$ 270. Jam: DPPH$^{\bullet}$ 180; $^{\bullet}$NO 2.45; $O_2^{\bullet}$ 50.3×10^6; HO$^{\bullet}$ 290	LPO, mg mL: jam 3.02	mg AAE/g: WE-F 2.12; ME-F 3.81; jam 6.41	[28]
20.	*S. wilfordii*; 70% ME of L	DPPH$^{\bullet}$: ME 6.01; DEf 3.67; EtAf 3.45; Buf 3.28; WR 9.04	TEAA: ME 2.24; DEf 2.97; EtAf 3.41; Buf 2.83; WR 1.51	ME 6.78; DEf 11.60; EtAf 12.55; Buf 10.99; WR 4.03	[37]
	S. wilfordii; 70% ME of L	DPPH$^{\bullet}$ 15.23	TEAA: L-0.86. LPO-86.92		[53]

DPPH$^{\bullet}$—2,2-diphenyl-1-picrylhydrazyl free radical scavenging capacity; ABTS$^{\bullet+}$—2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical cation decolouration assay; TEAA—trolox equivalent antioxidant activity, mmol/g; LPO—inhibition of lipid peroxidation, %; FRAP—ferric reducing antioxidant power. E—extract; f—fraction; R—residue; Et—ethanol; M—methanol; DE—diethyl ether; Bu—butanol; W—water; EtA—ethyl acetate; A—acetone; DCM—dichloromethane. I—inflorescences; L—leaves; F—fruits; S—stems; C—cortex, B—bark; fm—fresh mass. Unripe fruit (1), well matured on tree (2), matured for 1 week at room temperature (3), matured for 3 weeks at room temperature (4), fruit pulp from well matured fruits (5).

In order to identify the ability of different solvents to recover antioxidants from *S. aria* leaves various extracts were tested by DPPH$^{\bullet}$ scavenging method. Among the tested isolates, the EC_{50} value of ethyl acetate extract (2.99 mg/L), which contained 11.8% isoquercitrin, 6.0% astragalin, and 3.81% chlorogenic acid, was almost similar to reference antioxidant isoquercitrin, EC_{50} 2.76 mg/L [65]. Five strongly active constituents, namely isoquercitrin, rutin, quercetin 3-glucoside-7-rhamnoside, chlorogenic and neochlorogenic acids were found to be major components and principally responsible for the radical scavenging capacity of *S. aria* extracts [65]. Two interesting new coumarins, cashmins A (1) and B (2) were isolated from the methanolic extract of *S. cashmiriana*. Both compounds demonstrated outstanding antioxidant activity in H_2O_2 (IC_{50} 15.4 and 18.6 µmol/mL), as well as in ABTS$^{\bullet+}$ scavenging assays (IC_{50} 24.4 and 18.3 µmo/mL). For comparison, IC_{50} of ascorbic acid were 11.4 µmol/mL in H_2O_2 and 6.5 µmol/mL in ABTS$^{\bullet+}$ assays [15].

Bae et al. [69] tested the effects of treatment with carbohydrate hydrolases on the composition of TPC and flavonoids, as well as antioxidative activity of ethanol extract of dried *S. commixta* cortex. Amyloglucosidase, α-amylase, α-glucosidase and β-glucanase increased the contents of extractable polyphenols and flavonoids, as well as the DPPH$^{\bullet}$ scavenging capacity; particularly in case of applying β-glucanase [69].

Raudone et al. [71] detected twenty four constituents in the leaf samples of *S. anglica, S. aria, S. arranensis, S. aucuparia, S. austriaca, S. caucasica, S. commixta, S. discolor, S. gracilis, S. hostii, S. semi-incisa* and *S. tianschanica*, using ultra high performance liquid chromatography. Reducing activity of detected constituents was identified by the post-column FRAP assay; the highest total antioxidant activities of 175.3, 169.2 and 148.11 µmol TE/g dw were determined for *S. commixta, S. discolor* and *S. gracilis*, respectively.

Ethanol recovered antioxidants from *S. commixta* stems more effectively than hot water with the values of 504.39 and 364.64 µg/mg, respectively. Similarly, ethanol extracts demonstrated slightly higher antioxidant activities than water extracts in Fe^{2+} chelating, DPPH$^{\bullet}$, ABTS$^{\bullet+}$, hydroxyl and nitrite radical scavenging assays [68]. Extraction and fractionation of *S. domestica* fruits harvested at five different maturity stages gave the products with scavenging capacity in the range of 0.341–39.5 mg dwe/mg DPPH$^{\bullet}$ and the following order: water << dichloromethane < ethyl ether < ethyl acetate [58]. The fractions recovered with organic solvents possessed greater radical scavenging capacity than trolox, while the unripe fruits provided more antioxidants than the well-matured berries at room temperature. Finally, radical scavenging values strongly correlated with the total phenolic content in the fractions of *S. domestica* [58]. Olszewska et al. demonstrated that strong antioxidant fractions might be obtained from 70% methanol extracts of inflorescences and/or leaves of seven *Sorbus* spp. by using different solvents in a separatory funnel. *n*-Butanol and ethyl acetate gave the fractions with outstanding antioxidant capacity in the all applied assays: EC_{50} 3.2–5.2 µg/mL in DPPH$^{\bullet}$, 2.8–4.0 mmol TE/g in ABTS$^{\bullet+}$, 9.8–13.7 mmol Fe^{2+}/g in FRAP and 39.6–58.2% GAE in Folin-Ciocalteu [37]. Consequently, properly selected solvents may provide promising antioxidants for food and medicinal applications.

The fruits and jam of *S. aucuparia* and two varieties of *S. torminalis*, were assayed for DPPH$^{\bullet}$, $^{\bullet}$NO, HO$^{\bullet}$ and $O_2^{\bullet}$ scavenging capacity, FRAP, and Fe^{2+}/ascorbate induced LP inhibition. As already mentioned, *S. aucuparia* extracts were found to be the most effective almost in all tests, except for the assay toward the neutralisation of $O_2^{\bullet-}$ when *S. torminalis* var. *torminalis* was the most potent. *S. torminalis* var. *torminalis* and *semitorminalis* showed similar antioxidant activity, however, var. *torminalis* had a slightly better antiradical power towards $^{\bullet}$NO, $O_2^{\bullet-}$ and HO$^{\bullet}$, while the extracts of *semitorminalis* acted more effectively in scavenging DPPH$^{\bullet}$, inhibiting LP and reducing Fe^{2+} [28]. Antioxidant capacity of extracts depends also on the nature of the assay as well as the polarity of solvent. For example, the results of Bobinaitė et al. demonstrated superior antioxidant capacity of *S. aucuparia* pomace water extract in the all test systems: in DPPH$^{\bullet}$, FRAP and ORAC (oxygen radical absorbance capacity) assays it was 309 µmol TE/g, 323 µmol TE/g and 263 mg TE/g, respectively; while ethanol extract was the next with its DPPH$^{\bullet}$ and ORAC values of 103 µmol TE/g and 201 mg TE/g, respectively [59].

6. Toxic Constituents of Rowanberries

Parasorbic acid, an important inhibitor of germination, has been reported in the fruits and seeds of *S. aucuparia* at the level of 4–7 mg/g and 0.08–0.12 mg/g fw, respectively [79]. This compound irritates the gastric mucosa and, if consumed at larger amounts, can cause indigestion and kidney damage to humans. However, heat treatment or freezing modifies the parasorbic acid into nontoxic sorbic acid. Parasorbic acid is sensitive to changes of temperature and brakes into safe compounds if the berries are picked after the first frost [34]. The parasorbic acid is almost absent in the cultivated hybrids. The other toxic component in rowanberries is the cyanogenic glycoside prunasin, which is derived from the amino acid phenylalanine; 1 g of the prunasin can liberate 91.5 mg HCN (hydrogen cyanide). Thus, HCN from the seeds of rowanberries, when formed at the levels exceeding 2–3 mg/L, can cause respiratory failure and even death [80]. Therefore, while processing the rowanberry pomace, the separation of the seeds would be essential.

7. Promising Health Benefits and Related Applications in Foods, Nutraceuticals and Pharmaceuticals

It is evident that among phytochemicals and other nutrients, polyphenolic compounds and ascorbic acid may be considered as the most valuable health beneficial constituents, which have been reported in various anatomical parts of *Sorbus* spp. The polyphenolics, which may influence the colour and flavour, have demonstrated antioxidant [54,81], antidiabetic [11,82] anti-hyperlipidemic [83], anti-inflammatory [84], antimicrobial [85], anticancer [86,87] antiviral [67], antifungal [79], antitumoral [88], anti-periodontal [89], and anti-osteoarthritis [90] effects, as well as vasoprotective [84], neuroprotective [26,91,92], cardioprotective [36], hepatoprotective [7], properties and COX-2 (cyclooxygenase-2) inhibitory [93] activities. Many of these activities are correlated to antioxidant capacity of bioactive compounds, which at cellular level may neutralize excessive reactive oxygen species, and thereby protect important biomolecules in the conditions of oxidative stress, which can cause cellular injury and development of chronic diseases. Therefore, it has been hypothesized that antioxidant-rich diets might play an important role in neutralising the excessive reactive oxygen species [11]. This hypothesis and increasing amount of evidence in favour of it have encouraged many researchers to test many novel plant-based phytochemicals as natural candidates for developing health beneficial exogenous antioxidants. The other important role of antioxidants is to protect foods and other sensitive to oxidation products during processing and storage in order to extend their shelf life and improve the quality and safety [94]. Compared to pure synthetic compounds natural preparations of phenolic antioxidants can be more effective due to the synergistic effects of various molecules present in the plant-based products. In addition, natural ingredients are usually safer than their synthetic counterparts and therefore are preferred by the consumers [11].

The application of plant-based polyphenolic substances in lipid-containing foods, cosmetics, and medicinal products is hampered by their high polarity (hydrophilicity), which makes them poorly soluble in the lipid medium, which is composed mainly of triacylglycerols. Therefore, for increasing product lipophilicity some studies [94–96] applied derivatisation of phenolic compounds by attaching medium or long chain alkyl molecules. For instance, the lipophilised phenolic extract of *S. aucuparia* was more effective inhibitor of rapeseed oil oxidation during 7-day storage than the untreated one: it reduced peroxide value by 43% and improved the solubility of the phenolics during frying [95]. Hydrophilic fraction of rowanberry pomace contained most of the polyphenolic antioxidants, while lipophilic seed extracts could be beneficial as nutraceutical and cosmetic agents due to the high content of polyunsaturated fatty acids and carotenoids [97].

Water extracts of berries containing high amounts of low molecular weight proanthocyanidins, which were tested as the inhibitors of colon cancer-induced angiogenesis, turned out to be superior in reducing Caco-2 cell viability [59]. Due to the significant content of bioactive phenolics in fruits, the wild rowanberries inhibited lipid oxidation both in liposomes and in emulsions [31]. Aqueous methanol extracts of *S. aucuparia* fruits were potent antioxidants while the extracts of both

S. torminalis varieties, namely *torminalis* and *semitorminalis* effectively inhibited the growth of *E. coli*, var. *torminalis* being the best inhibitor of *Staphylococcus aureus* [28]. Polyphenols from two hybrid cultivars of *S. aucuparia*, Zoltaja and Granatnaja also delayed pathogenic *E. coli* growth. The phenolic extracts of wild rowanberries and cultivated breed Burka had an inhibitory effect on hemagglutination of *E. coli* HB101 (pRR7), which expresses the M hemagglutinin [60]. *S. aucuparia* berry extract isolated with acidified acetone also demonstrated high activity against *Salmonella enterica* ssp. *enterica* ATCC BAA-2162, and *Pseudomonas aeruginosa* ATCC 9027; it also exhibited moderate activity towards the two *Listeria monocytogenes* strains and *Proteus vulgaris* [66]. Water extract of *S. aucuparia* fruit inhibited the growth of Gram-positive *E. faecalis*, *S. aureus* and Gram-negative *S. enterica*, as well as the viability of *C. freundii* and *B. cereus* [59]. These findings prove that *S. aucuparia* extracts express strong antimicrobial activity against a wide scale of microorganisms and possess the high mitogenic activity expressed as the stimulating effect on hamster lymphocyte proliferation [66]. Water and methanol extracts of *S. aucuparia* fruits were also effective in the inhibition of acetylcholinesterase (AChE) and exhibited in vitro cytotoxicity in SRB assay, using tumour HeLa, MCF7 and HT-29 and healthy MRC-5 cell lines; however, they didn't exhibit selectivity towards tumour cell lines [28].

The content of chlorogenic acid in sweet rowanberries can reach up to 200 mg/100 g, which is comparable with Arabica variety coffee beans, the richest source of this phenolic acid containing 280 mg/100 g [31]. Chlorogenic acids have been associated with a decreased risk of type 2 diabetes (T2D); they hydrolyse to caffeic acid, which reduces glucose absorption and oxidative stress in vitro and inhibits glucose-6-phosphate translocase, thereby decreasing glucose output in the liver. Intact chlorogenic acids are poorly absorbed in the small intestine, while the released after hydrolysis cinnamic acids are effectively absorbed with the help of enzymes in the colon depending on the precursor chlorogenic acid type and individual characteristics of a person [98]. Consequently, caffeoylquinic acid derivatives isolated from *S. commixta* fruits might be used for the regulation of diabetic complications and other pathogenic complications. These compounds also showed the most potent inhibitory effect against formation of the advanced glycation end products (AGE); neo-chlorogenic, crypto-chlorogenic, and chlorogenic acid exhibited potent inhibitory effects against peroxynitrite in radical scavenging assay [99].

Boath et al. [100] have reported that *S. aucuparia* fruits inhibited α-glucosidase with IC_{50} value 30 µg GAE/mL and were as effective as the pharmaceutical inhibitor acarbose for maintaining post-prandial glycemic control in T2D. Lately berry extracts of 16 different *Sorbus* species of subgenus *Sorbus* and *Aria* were tested for their α-amylase and α-glucosidase inhibitory activity. The study included *S.aucuparia, S. aucuparia* f. *xanthocarpa,, S.commixta, S. commixta* var. *rufo-ferruginea, S. decora, S. discolor, S. hybrida, S. koehneana, S. vilmorinii* and crossbreeds *S. aucuparia* × *americana, Sorbus* × *meinichii, Sorbus* × *splendida* (from subgenus *Sorbus*) and *S. alnifolia, S. folgneri, S. latifolia, S. minima, S. norvegica* (from subgenus *Aria*). The berry extract of *S. norvegica*, which belongs to subgenus *Aria*, also inhibited α-amylase and α-glucosidase and therefore was used in an oral starch tolerance test in streptozotocin (STZ)-treated C57BL/6 mice; administration of 900 mg extract daily demonstrated anti-hyperglycemic activity, which however was 36 times lower than in case of clinically used acarbose [24]. Thus, the berries of *S. norvegica* (subgenus *Aria)* may have some prospects in management T2D.

Twenty-nine different extracts, fractions and residues obtained from *S. domestica* fruits, harvested at 5 maturity phases were assessed for their in vitro inhibitory capacity of a rate-limiting enzyme aldose reductase [36,47]. Diethyl ether and ethyl acetate fractions effectively inhibited aldose reductase and the effect was associated with the high content of flavonoids and hydroxycinnamic acid esters determined in the extracts by liquid chromatography with diode array detector and mass spectrometer (LC-DAD-MS). The authors concluded that consumption of *S. domestica* fruit might be a promising way to reduce the occurrence of long-term complications of T2D, particularly at the early disease stages. The fractions of diethyl ether, ethyl acetate and dichloromethane of *S. domestica* were also noted as potential antioxidants to be used in food and medicinal preparations [36,47]. In addition,

Bailie et al. [11] suggested that both flavonoids and terpenoids could offer benefits to treat a number of T2D symptoms.

Several studies reported the effects of *Sorbus* bioactives on cancer cell lines and some disease biomarkers. Thus, vanillic acid 4-*O*-α-L-rhamnopyranoside, protocatechuic acid anhydride and trivanilloyl-(1,3,4-trihydroxybenzoyl) ester, which were the predominant antioxidants of the *S. domestica* fruits, make it potentially useful for the mitigation of several diseases, such as *Clostridium difficile* infection, inflammatory bowel and irritable bowel syndrome [101]. Ethanolic *S. commixta* fruit extract remarkably reduced the viability of human lung cancer cell lines through the induction of apoptosis irrespective of their p53 status [102]. Another study reported that ethyl acetate fraction of *S. commixta* exhibited considerable inhibition against thrombin, prothrombin, blood coagulation factors and platelet aggregation, without haemolysis activity at the doses up to 0.5 mg/mL and therefore, has the potential to be used as a new anti-coagulation agent [103]. The juice of *S. sambucifolia* provoked differentiation of HL-60 leukemic cells to monocyte/macrophage characteristics in a concentration-dependent manner as denoted by histochemical and biochemical assays; it was suggested that these findings are promising for developing new agents suitable for differentiation therapy of leukaemia with fewer side effects [70]. The *S. umbellata* (Desf.) Fritsch var. *umbellata* leaf extract demonstrated dose-dependent cytotoxic effect to A549 and MCF-7 cells in MTT assay, while the highest cell proliferation inhibition was observed for the A549, 71.8% at 150 μg/mL [104].

Sakuranetin isolated from *S. commixta* plant actively inhibited rhinovirus-3 (HRV3) replication and at 100 μg/mL exhibited higher than 67% antiviral activity without cytotoxicity in epithelioid carcinoma cervix (HeLa) cells; hence, it may be promising in developing novel drugs for treating HRV3 infections [105]. Water extract of the dried *S. commixta* inner stem bark suppressed the production of $^{\bullet}$NO and prostaglandins at the transcriptional levels, thus acting as an anti-inflammatory remedy for ear oedema formation, which was induced by arachidonic acid in mouse. A targeted blockage of protein kinase B translocation and its upstream signalling pathways was suggested as a possible therapeutic approach to develop anti-inflammatory drugs in the treatment of chronic diseases [84]. Furthermore, lupeol isolated, from the stem bark of *S. commixta* showed significant inhibitory effects on osteoclastogenesis; therefore, addition of *S. commixta* and lupeol could be used for bone diseases, such as osteoporosis, Paget's disease, osteolysis associated with periodontal disease, and multiple myeloma [38].

The treatment of *S. commixta* cortexes by β-glucanase increased extract antioxidant activity, while its application resulted in the enhanced viability of human dermal fibroblasts exposed to ultraviolet (UV) light [69]. Furthermore, Kim et al. tested the leaf extract of *S. alnifolia*, among the others, to develop new natural cosmetic ingredients with antioxidant activity. As a result, the trials proved that the extract of *S. alnifolia* exhibited 87% inhibition of elastase activity when applied at 1 mg/mL. This result may provide the relevant application of plant-based inhibitors of general elastase in cosmetics with effects for UV-irradiated and dry skin [106].

The methanol extracts of the dried stems and twigs of *S. alnifolia* contributed for protection against chemically and genetically induced dopaminergic neurodegeneration. Moreover, methanol extract of *S. alnifolia* plant increased food-sensing functions in the dopaminergic neuron degraded worms by 58.4% hereby prolonging the average lifespan by 25.6%. Therefore, the extract of *S. alnifolia* can be a useful candidate for the treatment of Parkinson's disease [26].

8. Conclusions and Further Perspectives

Polyphenolic antioxidants are among the most popular topics in characterisation of different *Sorbus* spp. anatomical parts. Since *Sorbus* polyphenols (proanthocyanidins, chlorogenic acid isomers and flavonols) are recognized as potent antioxidants and health beneficial phytochemicals, and considering the significant phenolic content in various *Sorbus* spp., it can be concluded that their products could be an excellent sources of natural antioxidants [54]. Such bioactives may be useful both for protecting foods against oxidation/microbial spoilage and providing health benefits to

the consumers by incorporation of *Sorbus* preparations into functional foods, nutraceuticals and/or cosmeceuticals. The results of current review confirm the specific phenolic composition and antioxidant activity of different plant parts of numerous *Sorbus* spp. All parts of *S. commixta*, the fruit, leaves and inflorescences of *S. aria*, *S. aucuparia*, *S. sambucifolia*, the leaves and inflorescences of *S. gracilis* and *S. koehneana* and the leaves of *S. wilfordii* and *S. pogonopetala* may be distinguished as the materials demonstrating outstanding antioxidant effect.

However, more systematic studies are required for developing convenient and acceptable to consumer applications of *Sorbus* ingredients in foods and/or food supplements. Some studies have proved that the products of rowanberries demonstrate antioxidant activity and can be considered as a good source of antioxidants in the diet; however, these studies are not sufficient for wider applications of *Sorbus*. The production of functional foods and nutraceuticals from selected *Sorbus* spp. is envisaged to impart valuable biological effects, especially those related to immunity and health.

Author Contributions: V.S. collecting literature sources, writing—original draft preparation and editing; P.R.V. methodology, collecting literature sources, writing—review and editing, supervision; R.B. writing—review and editing, supervision. All authors have read and agreed to the published version of the article.

Funding: This research received no external funding.

Acknowledgments: This work is supported by VALORTECH project, which has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No 810630.

Conflicts of Interest: The authors declare no conflict of interests.

References

1. Vazquez-Flores, A.A.; Martinez-Gonzalez, A.I.; Alvarez-Parrilla, E.; Díaz-Sánchez, Á.G.; Rosa, L.A.; González-Aguilar, G.A.; Aguilar, C.N. Proanthocyanidins with a low degree of polymerization are good inhibitors of digestive enzymes because of their ability to form specific interactions: A hypothesis. *J. Food Sci.* **2018**, *83*, 2895–2902. [CrossRef] [PubMed]
2. Singh, J.; Metrani, R.; Shivanagoudra, S.R.; Jayaprakasha, G.K.; Patil, B.S. Review on bile acids: Effects of the gut microbiome, interactions with dietary fiber, and alterations in the bioaccessibility of bioactive compounds. *J. Agric. Food Chem.* **2019**, *67*, 9124–9138. [CrossRef] [PubMed]
3. Robertson, K.R.; Phipps, J.B.; Rohrer, J.R.; Smith, P.G. A synopsis of genera in Maloideae (Rosaceae). *Syst. Bot.* **1991**, *16*, 376–394. [CrossRef]
4. Sołtys, A.; Galanty, A.; Podolak, I. Ethnopharmacologically important but underestimated genus *Sorbus*: A comprehensive review. *Phytochem. Rev.* **2020**, *19*, 491–526. [CrossRef]
5. Poyrazoğlu, E.S. Changes in ascorbic acid and sugar content of rowanberries during ripening. *J. Food Qual.* **2004**, *27*, 366–370. [CrossRef]
6. Miletic, R.; Paunovic, S.M. Research into service tree (*Sorbus domestica* L.) population in eastern Serbia. *Genetika* **2012**, *44*, 483–490. [CrossRef]
7. Fomenko, S.E.; Kushnerova, N.F.; Sprygin, V.G.; Drugova, E.S.; Momot, T.V. Chemical composition and biological action of rowanberry extract. *Russ. J. Bioorg. Chem.* **2016**, *42*, 764–769. [CrossRef]
8. Hejcman, M.; Hejcmanová, P.; Pavlů, V.; Thorhallsdottirl, A.G. Nutritive value of leaf fodder from the main woody species in Iceland. In *Grassland Science in Europe, Vol. 19—EGF at 50: The Future of European Grasslands*; Hopkins, A., Collins, R.P., Fraser, M.D., King, V.R., Lloyd, D.C., Moorby, J.M., Robson, P.R.H., Eds.; European Grassland Federation EGF: Zürich, Switzerland, 2014; pp. 566–568.
9. USDA National Rersources Conservation Service. Available online: http://plants.usda.gov (accessed on 27 August 2020).
10. Ayupbek, A.; Hu, K.-L.; Aisa, H.A. Chemical constituents from the leaves of *Sorbus tianschanica*. *Chem. Nat. Compd.* **2012**, *48*, 133–134. [CrossRef]
11. Bailie, A.; Renaut, S.; Ubalijoro, E.; Guerrero-Analco, J.A.; Saleem, A.; Haddad, P.; Arnason, J.T.; Johns, T.; Cuerrier, A. Phytogeographic and genetic variation in *Sorbus*, a traditional antidiabetic medicine—Adaptation in action in both a plant and a discipline. *PeerJ* **2016**, *2*, 1–22. [CrossRef]

12. Mccune, L.M.; Johns, T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *J. Ethnopharmacol.* **2002**, *82*, 197–205. [CrossRef]
13. Moon, S.C.; Choi, H.J.; Chung, T.W.; Lee, J.H.; Lee, S.O.; Jung, M.H.; Kim, B.J.; Choi, J.Y.; Ha, K.T. *Sorbus commixta* water extract induces apoptotic cell death via a ROS-dependent pathway. *Oncol. Lett.* **2018**, *16*, 4193–4200. [CrossRef] [PubMed]
14. Bhatt, L.R.; Bae, M.S.; Kim, B.M.; Oh, G.S.; Chai, K.Y. A chalcone glycoside from the fruits of *Sorbus commixta* Hedl. *Molecules* **2009**, *14*, 5323–5327. [CrossRef] [PubMed]
15. Khan, S.; Fatima, I.; Kazmi, M.H.; Malik, A.; Afza, N.; Iqbal, L.; Latif, M. Cashmins A and B, potent antioxidant coumarins from *Sorbus cashmiriana*. *Chem. Nat. Compd.* **2015**, *51*, 626–629. [CrossRef]
16. Li, H.; Matsuura, M.; Li, W.; Li, Q.; Zhang, Q.; Koike, K. Chemical constituents from the fruits of *Sorbus pohuashanensis*. *Biochem. Syst. Ecol.* **2012**, *43*, 166–168. [CrossRef]
17. Francis, J.K. (Ed.) *Wildland Shrubs of the United States and Its Territories: Thamnic Descriptions: Volume 1*; Gen. Tech. Rep. IITF-GTR-26; U.S. Department of Agriculture, Forest Service, International Institute of Tropical Forestry: San Juan, PR, USA; U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: Fort Collins, CO, USA, 2004; 830p.
18. Olszewska, M. Separation of quercetin, sexangularetin, kaempferol and isorhamnetin for simultaneous HPLC determination of flavonoid aglycones in inflorescences, leaves and fruits of three *Sorbus* species. *J. Pharm. Biomed. Anal.* **2008**, *48*, 629–635. [CrossRef]
19. Gil-Izquierdo, A.; Mellenthin, A. Identification and quantitation of flavonols in rowanberry (*Sorbus aucuparia* L.) juice. *Eur. Food Res. Technol.* **2001**, *213*, 12–17. [CrossRef]
20. Vogl, S.; Picker, P.; Mihaly-Bison, J.; Fakhrudin, N.; Atanasov, A.G.; Heiss, E.H.; Wawrosch, C.; Reznicek, G.; Dirsch, V.M.; Saukel, J.; et al. Ethnopharmacological in vitro studies on Austria's folk medicine—An unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. *J. Ethnopharmacol.* **2013**, *149*, 750–771. [CrossRef]
21. Räty, M.; Caudullo, G.; de Rigo, D. Sorbus aucuparia in Europe: Distribution, habitat, usage and threats. In *European Atlas of Forest Tree Species*; San-MiguelAyanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A., Eds.; Publ. Off. EU: Luxembourg, 2016; pp. 176–177.
22. Plants for A Future. Available online: https://pfaf.org/user/DatabaseSearhResult.aspx (accessed on 23 July 2020).
23. Kalle, R.; Sõukand, R. Historical ethnobotanical review of wild edible plants of Estonia (1770s-1960s). *Acta Soc. Bot. Pol.* **2012**, *81*, 271–281. [CrossRef]
24. Broholm, S.L.; Gramsbergen, S.M.; Nyberg, N.T.; Jäger, A.K.; Staerk, D. Potential of *Sorbus* berry extracts for management of type 2 diabetes: Metabolomics investigation of 1H NMR spectra, α-amylase and α-glucosidase inhibitory activities, and in vivo anti-hyperglycaemic activity of *S. norvegica*. *J. Ethnopharmacol.* **2019**, *242*. [CrossRef]
25. Kovanda, M.; Challice, J. The genus *Micromeles* revisited. *Folia Geobot. Phytotaxon.* **1981**, *16*, 181–193. [CrossRef]
26. Cheon, S.M.; Jang, I.; Lee, M.H.; Kim, D.K.; Jeon, H.; Cha, D.S. *Sorbus alnifolia* protects dopaminergic neurodegeneration in *Caenorhabditis elegans*. *Pharm. Biol.* **2017**, *55*, 481–486. [CrossRef] [PubMed]
27. Aldasoro, J.J.; Aedo, C.; Garmendia, F.M. Revision of *Sorbus* subgenera *Aria* and *Torminaria* (Rosaceae-Maloideae). *Syst. Bot. Monogr.* **2004**, *69*, 1–148. [CrossRef]
28. Mrkonjić, Z.O.; Nađpal, J.D.; Beara, I.N.; Sabo, V.S.A.; Četojević-Simin, D.D.; Mimica-Dukić, N.M.; Lesjak, M.M. Phenolic profiling and bioactivities of fresh fruits and jam of *Sorbus* species. *J. Serbian Chem. Soc.* **2017**, *82*, 651–664. [CrossRef]
29. Sulusoglu, M. In vitro pollen viability and pollen germination of service tree (*Sorbus domestica* L.). *Int. J. Biosci.* **2014**, *5*, 108–114. [CrossRef]
30. Majić, B.; Šola, I.; Likić, S.; Cindrić, I.J.; Rusak, G. Characterisation of *Sorbus domestica* L. bark, fruits and seeds: Nutrient composition and antioxidant activity. *Food Technol. Biotechnol.* **2015**, *53*, 463–471. [CrossRef]
31. Hukkanen, A.T.; Pölönen, S.S.; Kärenlampi, S.O.; Kokko, H.I. Antioxidant capacity and phenolic content of sweet rowanberries. *J. Agric. Food Chem.* **2006**, *54*, 112–119. [CrossRef]

32. Sokolov, V.V.; Savel'ev, N.I.; Goncharov, N.P. I. V. Michurin's work on expansion of the plant horticulture assortment and improvement of food quality. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact Appl. Sci.* **2015**, *69*, 190–197. [CrossRef]
33. Rengarten, G.A.; Sorokopudov, V.N. Selection of rows as a decorative culture in Russia and in European countries. *Vestn. KrasGAU Agron.* **2019**, *6*, 9–15. (In Russian)
34. Mlcek, J.; Rop, O.; Jurikova, T.; Sochor, J.; Fisera, M.; Balla, S.; Baron, M.; Hrabe, J. Bioactive compounds in sweet rowanberry fruits of interspecific rowan crosses. *Cent. Eur. J. Biol.* **2014**, *9*, 1078–1086. [CrossRef]
35. Berna, E.; Kampuse, S.; Straumite, E. The suitability of different rowanberry cultivars for production of fruit marmalade. In Proceedings of the Annual 18th International Scientific Conference Research for Rural Development, Jelgava, Latvia, 16–18 May 2012; Treija, S., Skuja, I., Eds.; Latvia University of Agriculture: Jelgava, Latvia, 2012; Volume 1, pp. 109–116.
36. Acuña, U.M.; Atha, D.E.; Ma, J.; Nee, M.H.; Kennelly, E.J. Antioxidant capacities of ten edible North American plants. *Phyther. Res.* **2002**, *16*, 63–65. [CrossRef]
37. Olszewska, M.A.; Presler, A.; Michel, P. Profiling of phenolic compounds and antioxidant activity of dry extracts from the selected *Sorbus* species. *Molecules* **2012**, *17*, 3093–3113. [CrossRef] [PubMed]
38. Im, N.K.; Lee, D.S.; Lee, S.R.; Jeong, G.S. Lupeol isolated from *Sorbus commixta* suppresses 1α,25-(OH)2D3-mediated osteoclast differentiation and bone loss in vitro and in vivo. *J. Nat. Prod.* **2016**, *79*, 412–420. [CrossRef] [PubMed]
39. Kim, S.C.; Oh, J.; Subedi, L.; Kim, S.Y.; Choi, S.U.; Kang, R.L. Two new phenolic glycosides from *Sorbus commixta*. *Chem. Pharm. Bull.* **2018**, *66*, 839–842. [CrossRef] [PubMed]
40. Mikulic-Petkovsek, M.; Krska, B.; Kiprovski, B.; Veberic, R. Bioactive components and antioxidant capacity of fruits from nine *Sorbus* genotypes. *J. Food Sci.* **2017**, *82*, 647–658. [CrossRef] [PubMed]
41. Termentzi, A.; Kefalas, P.; Kokkalou, E. LC-DAD-MS (ESI+) analysis of the phenolic content of *Sorbus domestica* fruits in relation to their maturity stage. *Food Chem.* **2008**, *106*, 1234–1245. [CrossRef]
42. Connolly, B.A. × *Sorbaronia fallax* (Rosaceae): A new record of an intergeneric hybrid in Connecticut. *Rhodora* **2009**, *111*, 123–125. [CrossRef]
43. Pasko, P. South Siberian fruits: Their selected chemical constituents, biological activity, and traditional use in folk medicine and daily nutrition. *J. Med. Plants Res.* **2012**, *6*, 4698–4706. [CrossRef]
44. Velebil, J.; Businský, R. *Sorbus* × *thuringiaca*, the correct name for the diploid hybrid between *Sorbus aria* and *S. aucuparia* (Rosaceae). *Taxon* **2016**, *65*, 352–360. [CrossRef]
45. Termentzi, A.; Alexiou, P.; Demopoulos, V.J.; Kokkalou, E. The aldose reductase inhibitory capacity of *Sorbus domestica* fruit extracts depends on their phenolic content and may be useful for the control of diabetic complications. *Pharmazie* **2008**, *63*, 693–696. [CrossRef]
46. Yang, B.; Ahotupa, M.; Maeaettae, P.; Kallio, H. Composition and antioxidative activities of supercritical CO_2-extracted oils from seeds and soft parts of northern berries. *Food Res. Int.* **2011**, *44*, 2009–2017. [CrossRef]
47. Klavins, L.; Kviesis, J.; Steinberga, I.; Klavina, L.; Klavins, M. Gas chromatography-mass spectrometry study of lipids in northern berries. *Agron. Res.* **2016**, *14*, 1328–1346.
48. Niki, E.; Noguchi, N.; Tsuchihashi, H.; Gotoh, N. Interaction among vitamin C, vitamin E, and β-carotene. *Am. J. Clin. Nutr.* **1995**, *995*, 13225–13265. [CrossRef] [PubMed]
49. B Berņa, E.; Kampuse, S. The marmalades of sweet rowanberries as an example of a functional food. In Proceedings of the 7th International Congress of Food Technologists, Biotechnologists and Nutritionists, Opatija, Croatia, 20–23 September 2011; pp. 112–120.
50. Koushik, A.; Hunter, D.J.; Spiegelman, D.; Anderson, K.E.; Buring, J.E.; Freudenheim, J.L.; Goldbohm, R.A.; Hankinson, S.E.; Larsson, S.C.; Leitzmann, M.; et al. Intake of the major carotenoids and the risk of epithelial ovarian cancer in a pooled analysis of 10 cohort studies. *Int. J. Cancer* **2006**, *119*, 2148–2154. [CrossRef] [PubMed]
51. Aslantas, R.; Pirlak, L.; Güleryüz, M. The nutritional value of wild fruits from the North eastern Anatolia region of Turkey. *Asian J. Chem.* **2007**, *19*, 3072–3078.
52. Ivakhnov, A.D.; Sadkova, K.S.; Sobashnikova, A.S.; Skrebets, T.E. Optimization of oil extraction from rowanberry waste in alcoholic beverage production. *Russ. J. Phys. Chem. B* **2019**, *13*, 1135–1138. [CrossRef]
53. Olszewska, M.A.; Nowak, S.; Michel, P.; Banaszczak, P.; Kicel, A. Assessment of the content of phenolics and antioxidant action of inflorescences and leaves of selected species from the genus *Sorbus sensu stricto*. *Molecules* **2010**, *15*, 8769–8783. [CrossRef]

54. Olszewska, M.A.; Michel, P. Antioxidant activity of inflorescences, leaves and fruits of three *Sorbus* species in relation to their polyphenolic composition. *Nat. Prod. Res.* **2009**, *23*, 1507–1521. [CrossRef]
55. Becerra-Herrera, M.; Lazzoi, M.R.; Sayago, A.; Beltrán, R.; Del Sole, R.; Vasapollo, G. Extraction and determination of phenolic compounds in the berries of *Sorbus americana* Marsh and *Lonicera oblongifolia* (Goldie) Hook. *Food Anal. Methods* **2015**, *8*, 2554–2559. [CrossRef]
56. Gaivelyte, K.; Jakstas, V.; Razukas, A.; Janulis, V. Variation in the contents of neochlorogenic acid, chlorogenic acid and three quercetin glycosides in leaves and fruits of rowan (*Sorbus*) species and varieties from collections in Lithuania. *Nat. Prod. Commun.* **2013**, *8*, 1105–1110. [CrossRef]
57. Šavikin, K.P.; Zdunić, G.M.; Krstić-Milošević, D.B.; Šircelj, H.J.; Stešević, D.D. *Sorbus aucuparia* and *Sorbus aria* as a source of antioxidant phenolics, tocopherols, and pigments. *Chem. Biodivers.* **2017**, *14*, 1–11. [CrossRef]
58. Termentzi, A.; Kefalas, P.; Kokkalou, E. Antioxidant activities of various extracts and fractions of *Sorbus domestica* fruits at different maturity stages. *Food Chem.* **2006**, *98*, 599–608. [CrossRef]
59. Bobinaitė, R.; Grootaert, C.; Van Camp, J.; Šarkinas, A.; Liaudanskas, M.; Žvikas, V.; Viškelis, P.; Venskutonis, P.R. Chemical composition, antioxidant, antimicrobial and antiproliferative activities of the extracts isolated from the pomace of rowanberry (*Sorbus aucuparia* L.). *Food Res. Int.* **2020**, 109310. [CrossRef]
60. Kylli, P.; Nohynek, L.; Puupponen-Pimiä, R.; Westerlund-Wikström, B.; McDougall, G.; Stewart, D.; Heinonen, M. Rowanberry phenolics: Compositional analysis and bioactivities. *J. Agric. Food Chem.* **2010**, *58*, 11985–11992. [CrossRef] [PubMed]
61. Ekin, H.N.; Gokbulut, A.; Aydin, Z.U.; Donmez, A.A.; Orhan, I.E. Insight into anticholinesterase and antioxidant potential of thirty-four Rosaceae samples and phenolic characterization of the active extracts by HPLC. *Ind. Crops Prod.* **2016**, *91*, 104–113. [CrossRef]
62. Kim, M.B.; Park, J.S.; Lim, S. Bin Antioxidant activity and cell toxicity of pressurised liquid extracts from 20 selected plant species in Jeju, Korea. *Food Chem.* **2010**, *122*, 546–552. [CrossRef]
63. Rutkowska, M.; Olszewska, M.A.; Kolodziejczyk-Czepas, J.; Nowak, P.; Owczarek, A. *Sorbus domestica* leaf extracts and their activity markers: Antioxidant potential and synergy effects in scavenging assays of multiple oxidants. *Molecules* **2019**, *24*, 2289. [CrossRef]
64. Isaikina, N.V.; Kalinkina, G.I.; Razina, T.G.; Zueva, E.P.; Rybalkina, O.Y.; Ulirich, A.V.; Fedorova, E.P.; Shilova, A.B. *Sorbus aucuparia* L. fruit is a source of the drug for increasing the efficiency of tumor chemotherapy. *Russ. J. Bioorg. Chem.* **2018**, *44*, 899–905. [CrossRef]
65. Olszewska, M.A.; Michel, P. Activity-guided isolation and identification of free radical-scavenging components from various leaf extracts of *Sorbus aria* (L.) Crantz. *Nat. Prod. Res.* **2012**, *26*, 243–254. [CrossRef]
66. Denev, P.; Kratchanova, M.; Ciz, M.; Lojek, A.; Vasicek, O.; Nedelcheva, P.; Blazheva, D.; Toshkova, R.; Gardeva, E.; Yossifova, L.; et al. Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. *Food Chem.* **2014**, *157*, 37–44. [CrossRef]
67. Gu, H.; Chen, F.; Zhang, Q.; Zang, J. Application of ionic liquids in vacuum microwave-assisted extraction followed by macroporous resin isolation of three flavonoids rutin, hyperoside and hesperidin from *Sorbus tianschanica* leaves. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2016**, *1014*, 45–55. [CrossRef]
68. Yoo, J.-H.; Doh, E.-S.; Chang, J.-P.; Kil, K.-J. Antioxidant activities of hot water and ethanol extracts from the stem of *Sorbus commixta* Heal. *Korea J. Herbol.* **2017**, *32*, 29–36. [CrossRef]
69. Bae, J.-T.; Sim, G.-S.; Kim, J.-H.; Pyo, H.-B.; Yun, J.-W.; Lee, B.-C. Antioxidative activity of the hydrolytic enzyme treated *Sorbus commixta* Hedl. and its inhibitory effect on matrix metalloproteinase-1 in UV irradiated human dermal fibroblasts. *Arch. Pharm. Res.* **2007**, *30*, 1116–1123. [CrossRef] [PubMed]
70. Yoshizawa, Y.; Kawaii, S.; Urashima, M.; Fukase, T.; Sato, T.; Murofushi, N.; Nishimura, H. Differentiation-inducing effects of small fruit juices on HL-60 leukemic cells. *J. Agric. Food Chem.* **2000**, *48*, 3177–3182. [CrossRef] [PubMed]
71. Raudone, L.; Raudonis, R.; Gaivelyte, K.; Pukalskas, A.; Viškelis, P.; Venskutonis, P.R.; Janulis, V. Phytochemical and antioxidant profiles of leaves from different *Sorbus* L. species. *Nat. Prod. Res.* **2015**, *29*, 281–285. [CrossRef] [PubMed]

72. Rutkowska, M.; Owczarek, A.; Kolodziejczyk-Czepas, J.; Michel, P.; Piotrowska, D.G.; Kapusta, P.; Nowak, P.; Olszewska, M.A. Identification of bioactivity markers of *Sorbus domestica* leaves in chromatographic, spectroscopic and biological capacity tests: Application for the quality control. *Phytochem. Lett.* **2019**, *30*, 278–287. [CrossRef]
73. Ullah, H.; Wilfred, C.D.; Shaharun, M.S. Ionic liquid-based extraction and separation trends of bioactive compounds from plant biomass. *Sep. Sci. Technol.* **2019**, *54*, 559–579. [CrossRef]
74. Guerrero-Analco, J.A.; Martineau, L.; Saleem, A.; Madiraju, P.; Muhammad, A.; Durst, T.; Haddad, P.; Arnason, J.T. Bioassay-guided isolation of the antidiabetic principle from *Sorbus decora* (Rosaceae) used traditionally by the Eeyou Istchee Cree First Nations. *J. Nat. Prod.* **2010**, *73*, 1519–1523. [CrossRef]
75. Magnus, S.; Gazdik, F.; Anjum, N.A.; Kadlecova, E.; Lackova, Z.; Cernei, N.; Brtnicky, M.; Kynicky, J.; Klejdus, B.; Necas, T.; et al. Assessment of antioxidants in selected plant rootstocks. *Antioxidants* **2020**, *9*, 209. [CrossRef]
76. Kähkönen, M.P.; Hopia, A.I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 4076–4082. [CrossRef]
77. Saftner, R.; Polashock, J.; Ehlenfeldt, M.; Vinyard, B. Biochemical composition and antiradical activity of rowanberry (*Sorbus* L.) cultivars and hybrids with different Rosaceae L. cultivars. *Eur. J. Hortic. Sci.* **2009**, *59*, 195–201.
78. Baltacioğlu, C.; Velioğlu, S.; Karacabey, E. Changes in total phenolic and flavonoid contents of rowanberry fruit during postharvest storage. *J. Food Qual.* **2011**, *34*, 278–283. [CrossRef]
79. Raspé, O.; Findlay, C.; Jacquemart, A.L. *Sorbus aucuparia* L. *J. Ecol.* **2000**, *88*, 910–930. [CrossRef]
80. EFSA. Opinion of the scientific panel on contaminants in the food chain on a request from the Commission related to cyanogenic compounds as undesirable substances in animal feed. *EFSA J.* **2007**, *434*, 1–67.
81. Aladedunye, F.; Matthäus, B. Phenolic extracts from *Sorbus aucuparia* (L.) and *Malus baccata* (L.) berries: Antioxidant activity and performance in rapeseed oil during frying and storage. *Food Chem.* **2014**, *159*, 273–281. [CrossRef]
82. Vlavcheski, F.; Young, M.; Tsiani, E. Antidiabetic effects of hydroxytyrosol: In vitro and in vivo evidence. *Antioxidants* **2019**, *8*, 188. [CrossRef]
83. Zymone, K.; Raudone, L.; Raudonis, R.; Marksa, M.; Ivanauskas, L.; Janulis, V. Phytochemical profiling of fruit powders of twenty *Sorbus* L. Cultivars. *Molecules* **2018**, *23*, 2593. [CrossRef]
84. Yu, T.; Lee, Y.J.; Jang, H.J.; Kim, A.R.; Hong, S.; Kim, T.W.; Kim, M.Y.; Lee, J.; Lee, Y.G.; Cho, J.Y. Anti-inflammatory activity of *Sorbus commixta* water extract and its molecular inhibitory mechanism. *J. Ethnopharmacol.* **2011**, *134*, 493–500. [CrossRef]
85. Takó, M.; Kerekes, E.B.; Zambrano, C.; Kotogán, A.; Papp, T.; Krisch, J.; Vágvölgyi, C. Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. *Antioxidants* **2020**, *9*, 165. [CrossRef]
86. Rodríguez-García, C.; Sánchez-Quesada, C.; Gaforio, J.J.; Gaforio, J.J. Dietary flavonoids as cancer chemopreventive agents: An updated review of human studies. *Antioxidants* **2019**, *8*, 137. [CrossRef]
87. Kristo, A.S.; Klimis-Zacas, D.; Sikalidis, A.K. Protective role of dietary berries in cancer. *Antioxidants* **2016**, *5*, 37. [CrossRef]
88. Razina, T.G.; Zueva, E.P.; Ulrich, A.V.; Rybalkina, O.Y.; Chaikovskii, A.V.; Isaikina, N.V.; Kalinkina, G.I.; Zhdanov, V.V.; Zyuz'kov, G.N. Antitumor effects of *Sorbus aucuparia* L. extract highly saturated with anthocyans and their mechanisms. *Bull. Exp. Biol. Med.* **2016**, *162*, 93–97. [CrossRef] [PubMed]
89. Deligiannidou, G.E.; Papadopoulos, R.E.; Kontogiorgis, C.; Detsi, A.; Bezirtzoglou, E.; Constantinides, T. Unraveling natural products' role in osteoarthritis management—An overview. *Antioxidants* **2020**, *9*, 348. [CrossRef] [PubMed]
90. Varela-López, A.; Bullón, P.; Giampieri, F.; Quiles, J.L. Non-nutrient, naturally occurring phenolic compounds with antioxidant activity for the prevention and treatment of periodontal diseases. *Antioxidants* **2015**, *4*, 447–481. [CrossRef] [PubMed]
91. Magrone, T.; Magrone, M.; Russo, M.A.; Jirillo, E. Recent advances on the anti-inflammatory and antioxidant properties of red grape polyphenols: In vitro and in vivo studies. *Antioxidants* **2020**, *9*, 35. [CrossRef] [PubMed]
92. Grodzicki, W.; Dziendzikowska, K. The role of selected bioactive compounds in the prevention of Alzheimer's disease. *Antioxidants* **2020**, *9*, 229. [CrossRef]

93. Laube, M.; Kniess, T.; Pietzsch, J. Development of antioxidant COX-2 inhibitors as radioprotective agents for radiation therapy—A hypothesis-driven review. *Antioxidants* **2016**, *5*, 14. [CrossRef]
94. Bernini, R.; Carastro, I.; Palmini, G.; Tanini, A.; Zonefrati, R.; Pinelli, P.; Brandi, M.L.; Romani, A. Lipophilization of hydroxytyrosol-enriched fractions from *Olea europaea* L. byproducts and evaluation of the in vitro effects on a model of colorectal cancer cells. *J. Agric. Food Chem.* **2017**, *65*, 6506–6512. [CrossRef] [PubMed]
95. Aladedunye, F.; Niehaus, K.; Bednarz, H.; Thiyam-Hollander, U.; Fehling, E.; Matthäus, B. Enzymatic lipophilization of phenolic extract from rowanberry (*Sorbus aucuparia*) and evaluation of antioxidative activity in edible oil. *LWT Food Sci. Technol.* **2015**, *60*, 56–62. [CrossRef]
96. Zhong, Y.; Shahidi, F. Lipophilized epigallocatechin gallate (EGCG) derivatives as novel antioxidants. *J. Agric. Food Chem.* **2011**, *59*, 6526–6533. [CrossRef]
97. Bobinaitė, R.; Kraujalis, P.; Tamkutė, L.; Urbonavičienė, D.; Viškelis, P.; Venskutonis, P.R. Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents. *J. Ind. Eng. Chem.* **2020**, *85*, 152–160. [CrossRef]
98. Clifford, M.N.; Kerimi, A.; Williamson, G. Bioavailability and metabolism of chlorogenic acids (acyl-quinic acids) in humans. *Compr. Rev. Food Sci. Food Saf.* **2020**, 1–54. [CrossRef]
99. Kim, T.H. Chlorogenic acid isomers from *Sorbus commixta* of ulleung island origin and their inhibitory effects against advanced glycation end product (AGE) formation and radical scavenging activity. *J. Korean Soc. Food Sci. Nutr.* **2016**, *45*, 1208–1213. [CrossRef]
100. Boath, A.S.; Stewart, D.; McDougall, G.J. Berry components inhibit α-glucosidase in vitro: Synergies between acarbose and polyphenols from black currant and rowanberry. *Food Chem.* **2012**, *135*, 929–936. [CrossRef] [PubMed]
101. Küpeli Akkol, E.; Gürağaç Dereli, F.T.; Taştan, H.; Sobarzo-Sánchez, E.; Khan, H. Effect of *Sorbus domestica* and its active constituents in an experimental model of colitis rats induced by acetic acid. *J. Ethnopharmacol.* **2020**, *251*. [CrossRef] [PubMed]
102. Lee, T.K.; Roh, H.S.; Yu, J.S.; Kwon, D.J.; Kim, S.Y.; Baek, K.H.; Kim, K.H. A novel cytotoxic activity of the fruit of *Sorbus commixta* against human lung cancer cells and isolation of the major constituents. *J. Funct. Foods* **2017**, *30*, 1–7. [CrossRef]
103. Kim, M.S.; Sohn, H.Y. Anti-coagulation and anti-platelet aggregation activity of the mature fruit of *Sorbus commixta*. *Korean J. Microbiol. Biotechnol.* **2015**, *43*, 373–377. [CrossRef]
104. Kavak, D.D.; Akdeniz, B. *Sorbus umbellata* (Desf.) Fritsch var. *umbellata* leaves: Optimization of extraction conditions and investigation antimicrobial, cytotoxic, and β-glucuronidase inhibitory potential. *Plant Foods Hum. Nutr.* **2019**, *74*, 364–369. [CrossRef]
105. Choi, H.J. In vitro antiviral activity of sakuranetin against human rhinovirus 3. *Osong Public Heal. Res. Perspect.* **2017**, *8*, 415–420. [CrossRef]
106. Kim, M.J.; Jung, T.K.; Kim, M.-H.; Yoon, K.-S. In vitro screening of Jeju island plants for cosmetic ingredients. *KSBB J.* **2018**, *33*, 76–82. [CrossRef]

II

Sarv, V., Venskutonis, P.R., Rätsep, R., Aluvee, A., Kazernavičiūtė, R., Bhat, R. 2021. Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (*Sorbus aucuparia* L. L.) Cultivated in Estonia. *Antioxidants* 10, 1779: 1-15. doi.org/10.3390/antiox10111779

antioxidants

MDPI

Article

Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (*Sorbus aucuparia* L.) Cultivated in Estonia

Viive Sarv [1,2,*], Petras Rimantas Venskutonis [1,3], Reelika Rätsep [1,2], Alar Aluvee [1], Rita Kazernavičiūtė [3] and Rajeev Bhat [2]

[1] Polli Horticultural Research Centre, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Uus 2, Polli, Mulgi Parish, 69108 Viljandi, Estonia; rimas.venskutonis@ktu.lt (P.R.V.); reelika.ratsep@emu.ee (R.R.); Alar.Aluvee@emu.ee (A.A.)
[2] ERA Chair for Food (By-) Products Valorisation Technologies (VALORTECH), Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia; rajeev.bhat@emu.ee
[3] Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, LT-50254 Kaunas, Lithuania; rita.kazernaviciute@ktu.lt
* Correspondence: viive.sarv@emu.ee

Abstract: This study aimed to identify promising candidates of rowanberry cultivars for a wider cultivation and utilization. Antioxidant properties and phenolic content were evaluated for fruit, juice, and pomace samples of 16 different sweet rowanberry cultivars (cvs) and wild rowanberry (*S. aucuparia* L.), while the antioxidant potential was assessed using three different methods, based on the capacity to scavenge $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ and measure the oxygen radical absorbance capacity (ORAC). In general, the radical scavenging capacity was higher for hybrid cultivars, e.g., for cvs Likernaja, Burka, Granatnaja, and Rubinovaja in all assays. The highest value in the $ABTS^{\bullet+}$ assay was determined for the fruit sample Likernaja, and in $DPPH^{\bullet}$ assay in the pomace sample of cv. Likernaja, at 527.55 and 1068.28 µM TE/g dw, respectively. The highest ORAC value was found in the fruit sample of Burka (456.53 µM TE/g dw). Among the Nevezhino rowans, the highest radical scavenging values of all fractions were determined in cv. Solnechnaja. Regarding the total phenolic content (TPC), higher values were obtained in the whole fruits than in separated fractions, juice, and pomace. The tested hybrids had higher TPC values, either in fruit and pomace or in juice extracts, than those in the other analyzed *S. aucuparia* L. cultivars. While the fruit and juice samples showed higher anthocyanin (ACY) values, the pomace samples had higher hydroxycinnamic acid (HCA) contents on average. The results revealed that the different fractions of selected rowanberry cultivars can be a promising source of antioxidants and polyphenols for further potential applications. It is envisaged that the results of this study will serve in valorizing sweet rowanberry cultivars as value-added functional ingredients for food and non-food applications.

Keywords: antioxidants; polyphenolic compounds; rowanberry pomace; hybrid cultivars

Citation: Sarv, V.; Venskutonis, P.R.; Rätsep, R.; Aluvee, A.; Kazernavičiūtė, R.; Bhat, R. Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (*Sorbus aucuparia* L.) Cultivated in Estonia. *Antioxidants* **2021**, *10*, 1779. https://doi.org/10.3390/antiox10111779

Academic Editor: Catalina Alarcòn de-la-Lastra

Received: 28 September 2021
Accepted: 4 November 2021
Published: 6 November 2021

1. Introduction

According to the recent report by Grand View Research, Inc., the global market of polyphenols is predicted to reach USD 2.08 billion by 2025 [1]. These compounds have demonstrated antioxidant, anti-inflammatory, anti-diabetic, anti-diarrheal, anti-tumor, as well as diuretic and vasodilatory effects. Many fruits and particularly berries are superior sources of polyphenols with a high antioxidant capacity [2,3]. Therefore, fruit-origin raw materials have been growingly utilized to extract bioactive compounds for various applications. In some cases, the processing of fruits generates a substantial number of by-products [4]. For example, fruit pomace, which is a solid residue of juice pressing, consists mainly of skin, seeds, and pulp, and it accounts for approximately 10–35% of the mass of the initial fresh fruit [4]. Moreover, the pomace holds a considerable number of polyphenolic compounds, approximately 28–35% in the skin, 60–70% in seeds, and

10% in pulp, making it a potential source of natural antioxidants [5,6]. Although, many research articles have been published on the valorization of by-products from agro-industry, including fruit pomace [4], juice pressing residues of some fruit remain under-investigated.

Rowan is a fairly common fruit crop in different countries of the world. The orange or reddish fruits of *Sorbus aucuparia* L. are small (diameter 6–9 mm) and they have been traditionally used as diuretic, laxative, anti-inflammatory, and vasoprotective agents, against rheumatism and kidney diseases as well as for the treatment of various gastrointestinal and respiratory tract-related disorders [7].

Although the rowanberries have been used for juice, jams, or jellies [8,9], their application for foods is limited due to their bitter and astringent taste. To overcome this hindrance, the first sweet rowanberry clones were selected from the Sudety Mountains (Czech Republic) already in the 19th century. At the beginning of the 20th century, Russian scientist and plant breeder Michurin started a breeding program of sweet rowanberries for northern conditions and developed the most interesting group of *S. aucuparia* hybrids with *Pyrus, Malus, Aronia,* or *Crataegus* species [10]. The taste of the cultivated hybrid fruits such as Likernaja, Alaja Krupnaja, and Granatnaja (Figure 1), is less astringent, and the fruits are usually larger and darker in color than those of wild rowanberries [9,11]. The varieties Kubovaya, Zheltaya, and Krasnaya were selected from the sweet-fruited form of mboxemphS. aucuparia originated from the village Nevezhino in Russia, while the varieties Rossica and Rosina were bred of the Moravian mountain ash from the Sudety Mountains. Regarding the quality characteristics of rowanberries, Bussinka, Vefed, and Solnechnaja were rich in vitamin C content, while the latter two were also not astringent [12]. Moreover, previous investigations have reported the antioxidant capacity [3] and bacteriostatic effect [13] of both wild and cultivated rowanberry extracts.

Figure 1. Rowanberry cultivars 'Likernaja', 'Alaja Krupnaja' and 'Granatnaja'.

Considering the diverse genetic background of sweet rowanberry cultivars, there is no comprehensive information available about the antioxidant properties and phenolic content of these fruit, juice, and pomace. Therefore, the antioxidant capacity, phenolic content, and phytochemicals of 16 sweet rowanberry cultivars: cvs Burka, Alaja Krupnaja, Granatnaja, Kubovaja, Rosina, Rubinovaja, Angri, Bussinka, Likernaja, Moravica, Oranzhevaja, Krasnaja, Sahharnaja, Solnechnaja, Rossica, and Vefed, were determined using in vitro assays. It is expected that the results of this study will serve in valorizing sweet rowanberry cultivars as value-added functional ingredients for food and non-food applications.

2. Materials and Methods

The chemicals in procedures were analytical grade and purchased from Sigma-Aldrich (Steinheim, Germany).

2.1. Preparation of Sweet Rowanberry Samples

Ripe fruit of 16 sweet rowanberry cultivars (Table 1) and wild rowanberry were harvested in autumn 2019 from Polli Experimental Station, Estonia. All fruits were immediately frozen and stored at −20 °C. The low-speed juicer Smeg SJF01CREU (Smeg S.p.A, Guastalla, Italy) was used to extract the juice from defrosted fruit. The remaining pomace

accounted for approximately 15–20% of the weight of the fresh rowanberries. The rowanberries, juice, and pomace were frozen at −40 ± 2 °C and freeze-dried in an Advantage Plus Benchtop Freeze Dryer (SP Industries, Warminster, PA, USA) for 72 h at 30 µbar. The pomace samples were ground in a Retsch Mixer Mill M 400 (Haan, Germany) for 1.5 min at 30 Hz using ZrO_2 balls. The lyophilized rowanberries, pomace, and juice were stored in hermetically closed bottles at −25 °C.

Table 1. Description of selected sweet rowanberry *S. aucuparia* cultivars.

Cultivar	Origin	Breeding Background	Ref.
Burka	Russia, 1918	(*S. aria* × *Aronia arbutifolia* = *Sorbaronia alpina*) × *S. aucuparia*	[14,15]
Likernaja	Russia	S. *aucuparia* × *Aronia melanocarpa*	[15,16]
Granatnaja	Russia, 1925	*S. aucuparia* × *Crataegus sanguinea* = *Sorbocrataegus miczurinii*	[14,15]
Rubinovaja	Russia, 1927	*S. aucuparia* × *Pyrus communis* L.	[12,14,15]
Alaja Krupnaja	Russia, 1926	*S. aucuparia* × *Pyrus* sp. × *S. aucuparia* var. *moravica*	[14,15]
Moravica	Moravia, Check Republic, 19th cent.	The oldest cultivated *S. aucuparia*	[9,12]
Krasnaja	Nevezhino, Russia	Form of Nevezhino rowan (*S. aucuparia)*	[12,17]
Kubovaja	Nevezhino, Russia, 19th cent.	Form of Nevezhino rowan (*S. aucuparia)*	[12,14]
Oranzevaja	Nevezhino, Russia	Clone of Zheltaja, form of Nevezhino rowan (*S. aucuparia)*	[17]
Sahharnaja	Nevezhino, Russia	Form of Nevezhino rowan (*S. aucuparia)*	[12,17]
Vefed	Nevezhino, Russia	Cultivated form based on Nevezhino rowan (*S. aucuparia)*	[12]
Rossica	Germany, 1896	Clone of Moravica (*S. aucuparia)*	[12]
Solnechnaja	Nevezhino, Russia	Seedling of Kubovaja (*S. aucuparia)*	[12]
Angri	Nevezhino, Russia	Cultivated form based on Nevezhino rowan (*S. aucuparia)*	[12]
Bussinka	Nevezhino, Russia	Seedling of Kubovaja (*S. aucuparia)*	[12,15]
Rosina	Germany, 1946	Clone of Moravica (*S. aucuparia)*	[12,14]

2.2. Determination of Antioxidant Capacity

Antioxidant capacity was measured using four methods, based on the rowanberry phytochemicals to reduce Folin–Ciocalteu's reagent (generally called as total phenolic content, TPC), their ability to scavenge 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid radical cation ($ABTS^{\bullet+}$) and stable diphenyl-picrylhydrazyl radical ($DPPH^{\bullet}$), as well as oxygen radical absorbance capacity (ORAC). The experts have previously used at least 3 of these methods, including TPC and ORAC, for a comprehensive evaluation of the antioxidant potential of natural products [18]. All spectrophotometric measurements of juice and fruit samples and ORAC of pomace were performed on a FLUOstar Omega Microplate Reader (BMG Labtech, Offenburg, Germany); TPC and $ABTS^{\bullet+}$/$DPPH^{\bullet}$ scavenging values of pomace samples prepared by QUENCHER method were determined on a Spectronic Genesys 8 spectrophotometer (Thermo Spectronic, Rochester, NY, USA). TPC was expressed as gallic acid equivalents in grams of dry sample weight (mg GAE/g), radical scavenging in Trolox equivalents (mg TE/g) unless indicated differently. All described measurements in this section were replicated four times.

2.2.1. Sample Preparation

Freeze-dried juice samples were dissolved in methanol (MeOH, 1 w/v) by treating 15 min in the ultrasound bath. Then, the solutions were centrifuged at 4500 rpm for 5 min and transparent centrifugate was used directly for measurements. It was decided to apply the QUENCHER procedure for measuring the antioxidant capacity of freeze-dried fruit and pomace. This method enables the determination of antioxidant capacity both of bound and free radical scavengers [18]. However, grinding of the freeze-dried fruit was rather complicated, most likely due to the presence of viscous pectic substances; therefore, 1 g of fruit was homogenized with 10 mL of MeOH at 9500 rpm during 1 min in IKA T 25 digital ULTRA-TURRAX Disperser (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The homogenate was centrifuged at 12,000 rpm for 5 min and the supernatant was collected, diluted to the required concentration, and used for analysis.

The preparation of pomace samples in the QUENCHER procedure was carried out as described by Serpen et al. [18] with some modifications. The stock mixture was produced by mixing freeze-dried pomace with microcrystalline cellulose at a ratio of 1:1 (w/w). Afterward, a series of "solid dilutions" of stock mixture with microcrystalline cellulose was performed to obtain the concentrations in the range of 1–40 μg/mg. Based on these results, 10 mg of freeze-dried pomace were used in all assays.

2.2.2. Total Phenolic Content (TPC)

The TPC was measured with Folin–Ciocalteu's reagent as originally described by Singleton et al. [19]. Briefly, 30 μL of juice or fruits sample was mixed with 150 μL of 10-fold diluted with distilled water Folin−Ciocalteu reagent and 120 μL of 7.5% Na_2CO_3 in microplate wells. After mixing, the microplate was placed in the FLUOstar Omega Reader, shaken for 30 s, incubated for 30 min at room temperature, and the absorbance was read at 765 nm wavelength. All measurements were replicated four times. A blank sample, which was prepared daily, contained the same amount of distilled water. A series of gallic acid solutions in the concentration range of 0.025–0.35 mg/mL was used for the calibration curve (regression equation: $y = 9.8307x + 0.1215$, $R^2 = 0.9987$). In the case of pomace (QUENCHER approach) 10 mg of sample or cellulose (blank) were mixed with 150 μL of distilled H_20, 750 μL of Folin-Ciocalteu's reagent, and 600 μL of Na_2CO_3 solution, vortexed for 15 s, shaken at 250 rpm for 2 h in the dark, centrifuged (4500 rpm, 5 min) and the absorbance of optically clear supernatant was measured at 760 nm. Gallic acid solutions (150 μL) at various concentrations (0–80 μg/mL) were used for calibration.

2.2.3. DPPH$^\bullet$ Scavenging Capacity

DPPH$^\bullet$ scavenging capacity (RSC) of extracts was determined by a slightly modified spectrophotometric method of Brand-Williams et al. [20]. The aliquots of dissolved juice and fruits extracts (7.5 μL, 0.1%) were mixed in a FLUOstar Omega 96 well microplate reader with 300 μL of DPPH$^\bullet$. The decrease of absorbance was measured at 515 nm by comparing it with a blank. The final RSC values were calculated by using a regression equation $y = 275.34x + 5.4266$ ($R^2 = 0.99$), which was obtained by using different concentration solutions of Trolox for building the calibration curve. The antioxidant capacity of each sample is expressed as mg of Trolox equivalent (TE) per g of dry weight sample.

Pomace or cellulose (blank) were transferred to a centrifugation tube, mixed with 500 μL of MeOH and 1000 μL of working DPPH$^\bullet$ solution, vortexed for 15 s, shaken at 250 rpm for 2 h in the dark, centrifuged (4500 rpm, 5 min), and the absorbance of optically clear supernatant was measured at 515 nm. Trolox solutions (25 μL) at various concentrations (0–1500 μmol/L MeOH) were used for calibration. For each well, an aliquot of 7.5 μL (0.1%) sample was mixed with 300 μL of DPPH$^\bullet$. The decrease of absorbance was measured at 515 nm by comparing it with a blank sample.

2.2.4. $ABTS^{\bullet+}$ Scavenging Capacity

$ABTS^{\bullet+}$ decolorization assay was performed according to Re et al. [21], which is based on the reaction of $ABTS^{\bullet+}$ with antioxidants resulting in color change. The aliquots of dissolved juice and fruit extracts (3 µL, 0.1%) were mixed with 300 µL $ABTS^{\bullet+}$ solution in the microplate wells of FLUOstar Omega reader and the absorbance was measured at 734 nm against phosphate-buffered saline (PBS) solution, which was used as a blank. The final RSC values were calculated by using a regression equation $y = 99.766x + 2.4483$ ($R^2 = 0.99$).

Pomace or cellulose (blank) were mixed with 25 µL of MeOH and 1500 µL of working $ABTS^{\bullet+}$ solution, vortexed for 15 s, shaken at 250 rpm for 2 h in the dark, centrifuged (4500 rpm, 5 min), and the absorbance of optically clear supernatant was measured at 734 nm. Trolox solutions (25 µL) at various concentrations (0–1500 µmol/L MeOH) were used for calibration.

2.2.5. Oxygen Radical Absorbance Capacity (ORAC)

ORAC method was performed as described by Prior et al. [22] and Davalos et al. [23] by using fluorescein as a fluorescent probe. The stock solution of fluorescein was prepared according to Prior et al. [22]. The reaction was carried out in 75 mM phosphate buffer (pH 7.4), while the addition of antioxidant substances produced a more stable fluorescent signal which could reflect the antioxidant capacity.

For the subsequent assays, 25 µL (0.01%) of juice and fruit extract samples and 150 µL of PBS for fluorescein solution (95.68 nmol/L) were used. Solutions were placed in the 96 transparent flat-bottom microplate wells, the mixture was pre-incubated for 15 min at 37 °C, followed by rapid addition of AAPH solution as a peroxyl radical generator (25 µL; 240 mM) using a multichannel pipette. The microplate was immediately placed in the FLUOstar Omega reader, automatically shaken before each reading and the fluorescence was recorded every cycle (1 min × 1.1), a total of 120 cycles. The 485 nm excitation and 520 nm emission filters were used. At least three independent measurements were performed for each sample. Raw data were exported from the Mars software to Excel 2003 (Microsoft, Roselle, IL, USA) for further calculations. Antioxidant curves (fluorescence versus time) were normalized and from the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as:

$$AUC = (1 + f_5/f_0 + f_6/f_0 + f_7/f_0 + \cdots + f_i/f_0);$$

where f_0 = initial fluorescence reading at cycle 0, f_i = fluorescence reading at cycle i.

The final $ORAC_{FL}$ values were calculated by using a regression equation ($y = 0.1105x + 5.0662$, $R^2 = 0.98$) between Trolox concentration and AUC. The phosphate buffer saline (PBS) solutions of Trolox with known concentrations ranging from 5 to 250 µM/L were used for calibration. The antioxidant capacity in all assays is expressed as µM of Trolox equivalent (TE) per gram of dry weight sample.

Pomace or cellulose (blank) were mixed with 150 µL of PBS solution (75 mmol/L) and 900 µL of fluorescein solution (14 µmol/L PBS), vortexed for 15 s, shaken at 250 rpm for 60 min in the dark, and centrifuged (4500 rpm, 5 min). Optically clear supernatant (175 µL) was transferred to the 96-well black opaque microplates, pre-incubated for 15 min at 37 °C, followed by rapid addition of 25 µL of AAPH solution (240 mmol/L) as a peroxyl radical generator using a multichannel pipette. The fluorescence was recorded every cycle (1 min × 1.1), total of 90–140 cycles. Further experimental and data handling were the same as reported for extract analysis. Trolox solutions (150 µL) at various concentrations (0–500 µmol/L PBS) were used for calibration.

2.3. Identification and Quantification of Polyphenols by LC-MS Method

An ultra-high-performance liquid chromatography (UHPLC) was used for the analysis of individual phenolic compounds. Approximately 1 g of fresh fruit, juice, or pomace was

mixed in 10 mL of 50% ethanol acidified with 1% of HCl. Before analysis, the samples were homogenized for 3 min using the IKA Ultra-Turrax® Tube Drive (IKA®-Werke GmbH & Co. KG, Staufen, Germany) operating at 6000 rpm, followed by sonication at room temperature in an ultrasonic bath Branson 1800 (Emerson, St. Louis, MO, USA) for 15 min, and shaken in a multi-rotator Multi RS-60 (Biosan Sia, Riga, Latvia) for 30 min. Then, the samples were centrifuged at 13,000 rpm for 10 min (Eppendorf MiniSpin, rotor F-45–13.11) and 1 µL of extracts were pipetted into the vials for quantitative and qualitative chromatographic analysis, which was performed on UHPLC-DAD-LCMS 8040 (Shimadzu Nexera X2, Kyoto, Japan) using the reverse phase ACE Excel 3 C18-PFP column, 100 mm × 2.1 mm (ACE® Advanced Chromatography Technologies Ltd., Aberdeen, Scotland), and pre-column SecurityGuard ULTRA, C18 (Phenomenex, Torrance, CA, USA) operating at 40 °C for the separation of individual polyphenols. The UHPLC system was equipped with a binary solvent delivery pump LC-30AD, an autosampler Sil-30AC, column oven CTO-20AC and diode array detector SPD-M20A. The flow rate of the mobile phase was 0.25 mL/min, and the injected sample size was 1 µL. Acidified (1% formic acid) mobile phases consisted of Milli-Q water (A) and methanol (B). Separation was carried out for 40 min under the following conditions: gradient 0–27 min, 10–80% B; 27–29 min, 80–95% B; 29–35 min, isocratic 95% B, and re-equilibration of the system with 10% B 8 min before the next injection. All samples were kept at 4 °C during the analysis.

The calibration ranges of standards were adjusted considering the estimated concentrations of polyphenolic compounds in the samples. Individual phenolic compounds were identified by comparing their retention times, UV spectra, and parent and daughter ion masses (m/z) with those of the reference compounds. MS data acquisitions were performed on LCMS 8040 with the ESI source operating in both positive and negative modes. The interface voltage was set to 4.5 kV (both ESI+ and ESI−). Nitrogen was used as the nebulizing gas (3 L/min) and drying gas (15 L/min). The heat block temperature was 350 °C and the desolvation line (DL) temperature was 250 °C. All the samples were analyzed in triplicate, and the results were expressed as milligrams per gram of dry weight.

2.4. Statistics

The mean values and standard deviations (SD) of $ABTS^{\bullet+}$/$DPPH^{\bullet}$ radical scavenging capacity (RSC) results and total phenolic contents (TPC) were calculated using MS Excel and one-way analysis of the variance (ANOVA) at p value < 0.05. Correlation coefficients (R^2) between two RSCy assays and the polyphenolic groups were also calculated, using the statistical software from MS Excel.

3. Results and Discussions

3.1. Total Phenolic Content

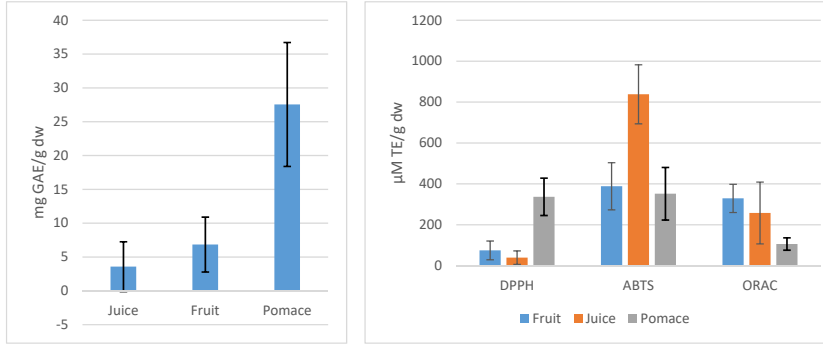
The results obtained for TPC are depicted in Figure 2a. Accordingly, the pomace fraction has the highest mean value of TPC: compared to the mean value of fruit, it is four-fold, while the mean value of fruit, in turn, is two times higher than the TPC of juice. The standard deviation (SD) bars demonstrate the variety of TPC among the 16 cvs. An especially wide range of TPC is among the pomace part of cvs. These findings prove that the pomace part obtained from specific cvs can provide us a valuable source of polyphenols for food and pharmaceutical purposes [4,24].

As demonstrated in Table 2, the TPC values of 16 sweet rowanberry cvs ranged between 2.53 and 15.05 mg GAE/g dw, 0.53 and 14.8 mg GAE/g dw, and 15.97 and 44.68 mg GAE/g dw for whole fruit, juice, and pomace fractions, respectively. The highest levels were found for all fractions of cvs Likernaja, Burka, Rubinovaja, and Granatnaja. The cvs Likernaja and Burka are the hybrids between rowanberry and chokeberry, S. *aucuparia* × *Aronia melanocarpa*, and *Sorbus aria* × *Aronia arbutifolia*, respectively; while Rubinovaja is × *Sorbopyrus* (S. *aucuparia* × *Pyrus*) and Granatnaja is × *Sorbocrataegus* (S. *aucuparia* × *Crataegus*). The pomace fractions of the hybrids demonstrated the TPC values of 44.68 mg GAE/g dw for cvs Burka and 41 mg GAE/g dw for Likernaja and Rubinovaja.

The TPC in the fruit of cv. Likernaja and cv. Burka was 15.05 and 14.78 mg GAE/g dw, respectively, while the contents in the juice of the same hybrids were 14.8 and 9.68 mg GAE/g dw, respectively. These results agree with the TPC values reported by Kampuse et al. [16] who found the highest TPC values for cv. Likernaja (484.9 mg/100 g fw) among the other 8 rowanberry cultivars. Hukkanen [14] tested many rowan cvs and found the highest TPC values for cvs Rubinovaja and Burka, 1014 and 820 mg/100 g of fw of fruit, respectively. In the research performed by Hukkanen et al., cv. Burka had the highest anthocyanin content among the sweet rowanberries. In the current research, the pomace fraction of cv. Moravica and wild rowanberry had very high TPCs, 29.32 and 31.7 mg GAE/g, respectively, while the highest TPCs among Nevezhino rowans were determined in the pomace of cv. Solnechnaja and Krasnaja, at 28.3 and 27.75 mg GAE/g dw, respectively. It may be observed that a significant fraction of polyphenols remains in the pomace, being the valuable part of rowanberries.

3.2. Antioxidant Capacity

The mean values of three antioxidant assays of rowanberry fruit, juice, and pomace (Figure 2b) demonstrate the different reaction mechanisms influencing these assays. Apak et al. [25] reported that although $ABTS^{\bullet+}$ reaction mechanism is still unclear, depending on individual antioxidants as well as reaction conditions, it is more a mixed-mode assay reagent, reacting by both ET (electron-) and HAT (hydrogen atom transfer) mechanisms. The $DPPH^{\bullet}$ is believed to act more like an H- atom acceptor, although the ET mechanism cannot be excluded, depending strongly on phenol-ionizing solvents and at alkaline pH where $DPPH^{\bullet}$ is a stable radical [25,26]. The ORAC assay is based on the HAT reaction mechanism [27].



(**a**) (**b**)

Figure 2. Mean values of TPC (**a**) and antioxidant capacity (**b**) of fruit, juice, and pomace of all cultivars in the current study.

Table 2. Total phenolic content, SET- and HAT-type antioxidant activity of fruit, juice, and pomace of 16 rowanberry genotypes and wild rowanberry.

	TPC			DPPH$^{\bullet}$			ABTS$^{\bullet+}$			ORAC		
	F	J	P	F	J	P	F	J	P	F	J	P
Bur	14.78 ± 1^{a}	9.68 ± 1^{b}	44.68 ± 2^{a}	127.8 ± 9^{b}	107.1 ± 4^{b}	522.3 ± 36^{a}	1010 ± 4^{b}	641.4 ± 3^{a}	576.8 ± 32^{a}	456.5 ± 33^{a}	435.7 ± 14^{ab}	125.3 ± 8^{abc}
Lik	15.05 ± 0^{a}	14.8 ± 0^{a}	41.31 ± 3^{b}	84.38 ± 6^{h}	125.61 ± 6^{a}	527.6 ± 33^{a}	1068 ± 8^{a}	615.1 ± 9^{a}	508.9 ± 27^{b}	416.5 ± 29^{ab}	381.9 ± 23^{de}	128.5 ± 4^{abc}
Gran	11.15 ± 1^{b}	5.79 ± 0^{c}	38.93 ± 3^{c}	177.5 ± 3^{a}	63.89 ± 4^{c}	402.7 ± 22^{c}	855.7 ± 1^{d}	500.1 ± 4^{b}	511.9 ± 35^{b}	399.4 ± 31^{cd}	396.8 ± 37^{cd}	133.1 ± 10^{abc}
Rub	9.51 ± 0^{c}	2.23 ± 0^{fg}	41.01 ± 4^{b}	110.2 ± 9^{c}	30.17 ± 2^{fg}	451.5 ± 36^{b}	990.1 ± 3^{b}	453.9 ± 4^{d}	584.2 ± 35^{a}	375.2 ± 5^{d}	335.2 ± 16^{e}	150.8 ± 3^{a}
Al K	6.46 ± 0^{de}	4.6 ± 0^{d}	20.73 ± 1^{i}	80.35 ± 8 i	58.85 ± 5^{d}	329.1 ± 23^{efg}	847.9 ± 5^{d}	351.3 ± 5^{g}	371.5 ± 19^{c}	266.4 ± 18^{gh}	413.4 ± 23^{bc}	66.52 ± 1^{gh}
Mor	6.54 ± 0^{de}	0.53 ± 0^{i}	29.32 ± 2^{e}	87.54 ± 5^{g}	18.06 ± 1^{i}	330.9 ± 16^{efg}	770.1 ± 5^{f}	123.2 ± 1^{j}	179.9 ± 11^{g}	299.3 ± 18^{f}	23.81 ± 1^{hi}	106.0 ± 2^{bcdef}
Kras	2.53 ± 0^{h}	1.33 ± 0^{h}	27.75 ± 2^{f}	39.03 ± 3^{l}	14.06 ± 1^{j}	268.6 ± 12^{hi}	801.4 ± 5^{e}	133.9 ± 2^{j}	228.1 ± 14^{f}	243.8 ± 9^{h}	393.7 ± 27^{cd}	99.64 ± 6^{cdefg}
Kub	2.57 ± 0^{h}	1.03 ± 0^{hi}	24.81 ± 1^{g}	43.71 ± 3^{j}	30.52 ± 1^{fg}	286.9 ± 24^{h}	699.8 ± 5^{h}	283.1 ± 4^{h}	329.1 ± 29^{d}	256.4 ± 15^{h}	388.2 ± 34^{cd}	80.03 ± 7^{efg}
Oranz	2.84 ± 0^{gh}	1.16 ± 0^{h}	19.76 ± 1^{j}	40.67 ± 3^{k}	33.08 ± 2^{fg}	172.1 ± 12^{j}	666.0 ± 1^{i}	247.4 ± 4^{i}	180.4 ± 5^{g}	239.1 ± 9^{h}	53.10 ± 3^{h}	43.87 ± 1^{h}
Sahh	5.77 ± 0^{e}	3.58 ± 0^{e}	25.37 ± 2^{g}	15.10 ± 1^{p}	33.32 ± 2^{f}	263.2 ± 15^{hi}	756.1 ± 5^{fg}	260.2 ± 5^{hi}	396.0 ± 8^{c}	293.85 ± 1^{fg}	209.2 ± 16^{f}	110.7 ± 10^{bcde}
Vef	7.33 ± 0^{d}	1.24 ± 0^{h}	15.97 ± 1^{l}	25.17 ± 1^{n}	20.18 ± 1^{hi}	317.6 ± 24^{fg}	913.7 ± 7^{c}	416.0 ± 7^{ef}	209.9 ± 16^{fg}	313.0 ± 10^{e}	19.70 ± 1^{i}	75.43 ± 7^{fgh}
Ross	4.45 ± 0^{f}	2.1 ± 0^{g}	18.61 ± 1^{k}	109.5 ± 5^{d}	6.15 ± 0^{k}	244.7 ± 18^{i}	813.0 ± 4^{e}	395.3 ± 2^{ef}	293.1 ± 24^{e}	380.4 ± 16^{cd}	122.1 ± 2^{g}	79.39 ± 2^{bcdef}
Soln	8.64 ± 0^{c}	3.8 ± 0^{e}	28.3 ± 2^{f}	91.73 ± 6^{f}	32.36 ± 1^{g}	324.5 ± 23^{efg}	911.5 ± 7^{c}	420.9 ± 4^{ef}	321.9 ± 23^{de}	406.5 ± 8^{bc}	443.7 ± 39^{a}	146.6 ± 9^{a}
Ang	3.77 ± 0^{g}	2.65 ± 0^{f}	23.02 ± 2^{h}	21.89 ± 2^{o}	31.64 ± 1^{fg}	286.9 ± 23^{h}	728.9 ± 3^{gh}	470.2 ± 3^{cd}	297.2 ± 5^{e}	329.4 ± 25^{e}	215.5 ± 4^{f}	117.5 ± 11^{abcd}
Buss	2.81 ± 0^{gh}	3.5 ± 0^{e}	16.04 ± 1^{l}	108.2 ± 5^{e}	37.56 ± 1^{e}	297.8 ± 22^{g}	756.5 ± 4^{fg}	420.2 ± 3^{e}	369.6 ± 25^{c}	259.4 ± 10^{h}	119.6 ± 8^{g}	84.62 ± 7^{defg}
Rosi	5.29 ± 0^{f}	1.1 ± 0^{h}	21.12 ± 1^{i}	31.60 ± 2^{m}	6.03 ± 0^{k}	332.5 ± 15^{ef}	803.6 ± 8^{e}	389.9 ± 2^{f}	300.2 ± 14^{de}	329.3 ± 28^{e}	203.2 ± 12^{f}	116.4 ± 8^{abcd}
Wild	NA	1.49 ± 0^{h}	31.7 ± 2^{d}	NA	21.77 ± 1^{h}	358.6 ± 24^{e}	NA	470.7 ± 5^{c}	313.2 ± 19^{de}	NA	226.9 ± 16^{f}	135.2 ± 4^{ab}

Results are mean values of four replicate analyses calculated in mg GAE/g dw for TPC and μM TE/g dw for antioxidant capacity. NA—data not available; different letters on columns mark significant differences at $p \leq 0.05$.

The $ABTS^{\bullet+}$/$DPPH^{\bullet}$ scavenging and ORAC values are presented in Table 2. The $DPPH^{\bullet}$ scavenging activity ranged from 15.1 to 177.5 μM TE/g dw, 6.03 to 125.6 μM TE/g dw, and 172.1 to 527.6 μM TE/g dw, for fruit, juice, and pomace, respectively. Using $ABTS^{\bullet+}$ assay the antioxidant capacity values were between 666 and 1068 μM TE/g dw, 123.2 and 641.4 μM TE/g dw, and 179.9 and 584.2 μM TE/g dw for fruit, juice, and pomace, respectively. The results of ORAC assay ranged from 239.1 to 456.5 μM TE/g dw, 19.7 to 443.7 μM TE/g dw, and 43.87 to 150.8 μM TE/g dw, for fruit, juice, and pomace, respectively. All fractions of cvs Likernaja, Burka, Rubinovaja, and Granatnaja had the antiradical capacity values above the average. Comparing the pomace fractions, the cv. Likernaja presented the highest $DPPH^{\bullet}$ value of 527.55 μM TE/g dw, the cv. Burka had the highest $ABTS^{\bullet+}$ value of 576.77 μM TE/g dw, and the cv. Rubinovaja demonstrated the highest ORAC value of 150.75 μM TE/g dw. From previous studies, Jurikova et al. [16] and Kampuse et al. [28] found the highest antioxidant activity of cv. Likernaja which is among the other hybrids. Compared to the other cvs, all fractions of cv. Solnechnaja had very high ORAC values, as well as the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ values were above the average of 17 pomace samples. While the average ORAC and $ABTS^{\bullet+}$ values raise in the direction: pomace < juice < fruit, the rise of $DPPH^{\bullet}$ values is juice < fruit < pomace, and the average fruit and juice values of $ABTS^{\bullet+}$ are 10-fold compared to $DPPH^{\bullet}$ values. This phenomenon can be explained by the different reaction mechanisms in $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and ORAC assays.

3.3. Identification and Quantification of Individual Phenolic Compounds in Different Fractions of Sweet Rowanberry Cultivars

The extracts recovered with acidified ethanol from fruit, juice, and pomace fractions were analyzed by UHPLC-DAD-MS/MS. The results (Figure 3 and Table 3) revealed that sweet rowanberry cvs are rich in caffeoylquinic acids, especially chlorogenic and neochlorogenic acids, ranging between 1.07 and 4.59 mg/g dw and between 0.75 and 6.13 mg/g dw, respectively. In our experiment, the highest contents of neochlorogenic acid were found in the fruit and juice samples of cvs Likernaja, Burka, Granatnaja, and Rubinovaja. The highest chlorogenic acid contents were determined in the fruit and juice samples of cvs Sahharnaja, Bussinka, Angri, and wild rowanberry. The neochlorogenic acids followed by chlorogenic acids were the most dominant phenolic acids in pomace samples (Figure 3). These findings were similar to the previous study of Bobinaitė et al. [29]. In the current study, the highest contents of neochlorogenic acid were tested in cvs Likernaja and Solnechnaja, but relatively high contents were determined also in cvs Burka, Bussinka and Granatnaja. Comparative data were reported by Jurikova et al., who found the highest content of chlorogenic acid in cvs Likernaja (100.9 mg/100 g fw) and Granatnaja (90.62 mg/100 g fw) [16]. While testing the chlorogenic acid content of the pomace samples, the highest values were found for wild rowanberry and cvs Bussinka and Sahharnaja, at 4.79 mg/g dw, 3.64 mg/g dw, and 3.62 mg/g dw, respectively. Mikulic-Petkovsek et al. [30] also reported cv. Bussinka to be rich in neochlorogenic acid.

Anthocyanins were the second most abundant group of polyphenols in sweet rowanberry cultivars. For instance, the fruits of cv. Burka had an even higher total ACY content (7.27 mg/g dw) than the content of total hydroxycinnamic acids (HCA), 5.10 mg/g dw. The other rowanberry hybrids, such as cvs Likernaja, Granatnaja, and Rubinovaja, also had relatively high total content of ACY, 6.33 mg/g dw, 3.20 mg/g dw, and 2.28 mg/g dw, respectively. The major part of ACY in the fruit and juice samples of hybrids was cyanidin-3-galactoside (up to 91% for Rubinovaja), followed by cyanidin-3-arabinoside (up to 21–22% for Likernaja and Burka). Cyanidin glucosides are a common group of anthocyanins in the rowanberries [30]. Kylli et al. [13] and Hukkanen et al. [14] also reported high contents of cyanidin-3-galactoside and cyanidin-3-arabinoside (together > 90% of the total ACYs) in the rowanberry hybrids. The fruit and juice of the other rowanberry cvs had ACY contents of less than 1 mg/g dw. Interestingly, in the case of rowanberry pomace, cyanidin-3-glucoside was the major part (up to 97%) of ACYs. Zymone et al. [15] and Mikulic-Petkovsec et al. [30] found cyanidin-3-galactoside to be the predominant

anthocyanin in rowanberry pomace powder fruits. In our study, the highest total content of ACYs was found in pomace of cvs Burka and Likernaja, followed by cvs Rubinovaja and Granatnaja. The latter two are hybrid cultivars, originating from sweet rowanberries with intense dark colors.

The average content of ACYs was found up to 10-fold in the fruit and juice samples compared to that in pomace samples. At the same time, the average content of flavanols in the pomace samples was up to 4.8 times higher than that in the juice and fruit samples. In addition, the average contents of flavanols were lower in the fruit and juice samples than in the pomace samples.

A principal component analysis (PCA) of eight major phenolic compounds (Ncha, ChA, Cygal, Cyglu, Cyara, Qgal, Qglu, and Qrut) was conducted for the rowanberry fruit, juice, and pomace samples (Figure 4). All three (a, b, c) plots differentiated the cvs into two color-based groups, e.g., dark red hybrid cvs group (blue) and orange group of all other sweet rowanberry cvs (red). The first (a) plot, which illustrates the differentiation of fruit samples, had the highest score of two factors 79.54%, while the plot score of two factors for juice and pomace samples were 73.62% and 69.89%, respectively. The dark red hybrid samples have remarkably higher ACY content than the orange cvs, therefore, five hybrid samples located far from the 0-point of principal components, while most of orange-colored samples located nearby the 0-point of principal components due to more similar phytochemical compositions of these fruit.

Selecting the cvs with the best yield (years 2019 and 2021) and antioxidant capacity, four potential cvs among sixteen emerged. Therefore, hybrid cvs Likernaja and Burka, as well as Nevezhino rowans Sahharnaja and Solnechnaja, but also the wild rowanberry will be used in the further studies.

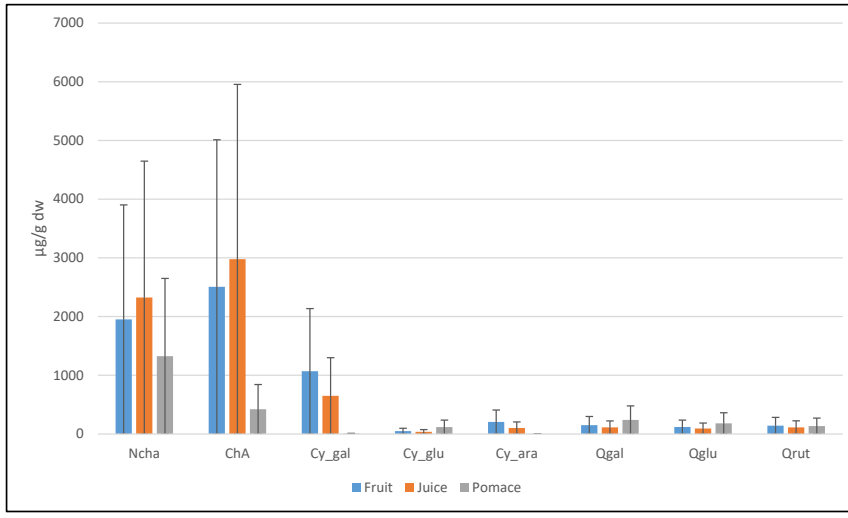
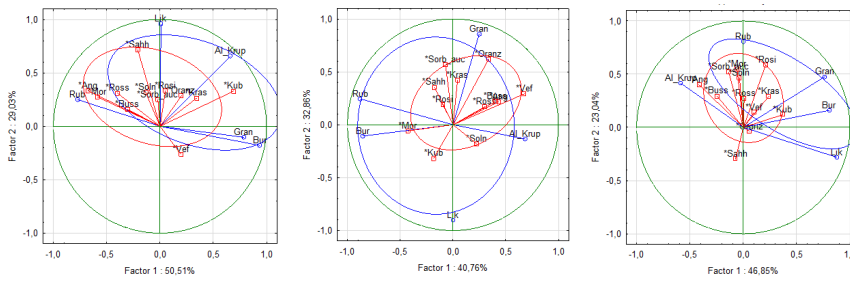


Figure 3. The mean contents of major polyphenolic compounds for all cultivars in current study.

Table 3. The distribution of individual phenolic compounds (μg/g dw) in fruit, juice, and pomace extracts of 17 sweet rowanberry cultivars.

		Bur	Lik	Gran	Rub	Al_K	Mor	Kras	Kub	Oranz	Sahh	Vef	Ross	Soln	Ang	Buss	Rosi	Wild
NCha	F	3086 ± 781	4955 ± 323	2553 ± 182	2441 ± 231	1991 ± 132	1677 ± 131	1014 ± 164	991 ± 0.82	0.944 ± 0.073	1891 ± 141	1779 ± 162	1181 ± 103	2541 ± 182	1241 ± 122	1850 ± 183	1518 ± 142	1531 ± 101
	J	3930 ± 123	6127 ± 108	3497 ± 42	3402 ± 133	2475 ± 46	1830 ± 28	1122 ± 19	1176 ± 42	1023 ± 46	2122 ± 55	1963 ± 122	1289 ± 14	2040 ± 692	1461 ± 29	2297 ± 27	1942 ± 28	1813 ± 41
	p	1681 ± 61	2172 ± 182	1621 ± 50	1453 ± 92	1181 ± 43	1171 ± 21	862 ± 35	752 ± 21	713 ± 12	1281 ± 43	1472 ± 32	901 ± 12	2021 ± 44	981 ± 22	1641 ± 21	1204 ± 42	1392 ± 11
ChA	F	2013 ± 502	2269 ± 83	2011 ± 2.1	2440 ± 72	1052 ± 43	2662 ± 61	2636 ± 12	2700 ± 121	2448 ± 41	3789 ± 83	1982 ± 41	2692 ± 52	2028 ± 32	3031 ± 63	3142 ± 102	2450 ± 52	3312 ± 44
	J	2213 ± 59	2640 ± 59	2731 ± 102	3368 ± 140	1265 ± 60	2834 ± 216	3139 ± 145	3186 ± 126	2625 ± 122	4595 ± 106	2202 ± 147	3064 ± 126	2515 ± 129	3459 ± 76	3843 ± 209	3359 ± 95	3591 ± 154
	p	1564 ± 28	1620 ± 102	1994 ± 64	2261 ± 135	1070 ± 18	2745 ± 115	3232 ± 27	2782 ± 216	2669 ± 69	3622 ± 59	2205 ± 31	2894 ± 91	2477 ± 81	3161 ± 123	3639 ± 93	3024 ± 23	4782 ± 181
Cyga1	F	5526 ± 602	4775 ± 263	2661 ± 122	2077 ± 73	716 ± 12.	274 ±14	137 ± 20	158 ± 12	81 ± 1	118 ± 12	290 ± 13	132 ± 21	109 ± 12	147 ± 1	506 ±22	279 ± 12	183 ± 1
	J	2627 ± 184	2704 ± 413	1884 ± 63	1288 ± 92	497 ± 13	235 ±10	115 ± 4	146 ± 7	51 ± 12	94 ± 7	227 ± 20	126 ± 5	113 ± 8	134 ± 7	460 ±13	273 ± 8	72 ± 12
	p	28 ± 2	26 ± 2	17 ± 2	9 ± 0	5 ± 0	2 ± 0	1 ± 0	1 ± 0	0	1 ± 0	2 ± 0	1 ± 0	1 ± 0	1 ± 0	3 ± 0	1 ± 0.0	2 ± 0
Cyglu	F	217 ± 42	175 ± 32	119 ± 11	141 ± 13	10 ± 2	18 ± 2	0	1 ± 0	0	0	10	1 ± 0	1 ± 0	1 ± 0	121 ± 2	25 ± 2	1 ± 0
	J	126 ± 4	127 ± 2	78 ± 1	110 ± 2	6 ± 0	18 ± 0	1 ± 0	1 ± 0	0	1 ± 0	1 ± 0	32 ± 2	1 ± 0	0	113 ± 2	23 ± 2	1 ± 0
	p	600 ± 31	554 ± 21	323 ± 53	362 ± 32	18 ± 2	27 ± 1	2 ± 0	66 ± 2	1 ± 2	1 ± 0	5 ± 0	2 ± 0	2 ± 0	2 ± 0	6 ± 0	36 ± 2	14 ± 2
Cyara	F	1538 ± 21	1380 ± 74.	424 ± 2.	60 ± 2	13 ± 2	5 ± 0	6 ± 0	6 ± 0	2 ± 0	4 ± 0	14 ± 1	5 ± 0	3 ± 0.0	6 ± 1	11 ± 2	5 ± 0	6 ± 0
	J	668 ± 57	690 ± 130	288 ± 8	37 ± 2	8 ± 0	3 ± 0	4 ± 0	5 ± 0	1 ± 0	3 ± 0	11 ± 2	4 ± 0	3 ± 0	5 ± 0	9 ± 0	4 ± 0	2 ± 0
	p	9 ± 0	8 ± 0	4 ± 0	1 ± 0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ecat	F	27 ± 1	31 ± 2	27 ± 2	26 ± 2	22 ± 2	17 ± 1	28 ± 3	35 ± 2	27 ± 6	20 ± 2	13 ± 2	44 ± 2	13 ± 2	38 ± 6	13 ± 0	15 ± 2	33 ± 2
	J	10 ± 1	14 ± 1	16 ± 1	12 ± 1	11 ± 1	8 ± 1	16 ± 1	24 ± 1	14 ± 1	13 ± 0.1	8 ± 0.1	23 ± 0.1	8 ± 0.1	20 ± 0.1	07 ± 0	10 ± 0	12 ± 0
	p	59 ± 3	62 ± 3	69 ± 1	65 ± 3	65 ± 3	59 ± 2	70 ± 7	102 ± 3	84 ± 5	96 ± 3	65 ± 5	103 ± 1	54 ± 6	100 ± 2	53 ± 2	60 ± 3	111 ± 3
Cat	F	9 ± 2	9 ± 2	11 ± 2	26 ± 2	17 ± 2	19 ± 2	18 ± 2	22 ± 02	30 ± 2	19 ± 2	5 ± 2	23 ± 2	22 ± 2	25 ± 02	92	16 ± 2	17 ± 2
	J	5 ± 0	6 ± 0	8 ± 1	13 ± 2	8 ± 2	10 ± 1	12 ± 1	014 ± 1	016 ± 1	14 ± 1	3 ± 1	12 ± 1	14 ± 1	12 ± 1	6 ± 1	12 ± 1	8 ± 1
	p	21 ± 2	22 ± 2	37 ± 2	65 ± 2	48 ± 2	69 ± 2	44 ± 2	54 ± 2	82 ± 2	90 ± 2	22 ± 2	59±	93 ± 2	56 ± 0	29 ± 2	66 ± 2	52 ± 0
Coum	p	12 ± 1	9 ± 1	8 ± 1	24 ± 1	4 ± 0	5 ± 0	7 ± 1	9 ± 1	7 ± 1	6 ± 1	15 ± 1	15 ± 1	9 ± 1	17 ± 1	12 ± 1	8 ± 1	21 ± 1
Fer	p	10 ± 0	11 ± 0	8 ± 0	28 ± 0	4 ± 0	5 ± 0	7 ± 0	9 ± 0	5 ± 0	6 ± 0	23 ± 0	16 ± 0	7 ± 0	20 ± 0	25 ± 0	8 ± 0	10 ± 0
Q	F	50 ± 1	9 ± 1	9 ± 1	9 ± 1	5 ± 0	8 ± 1	6 ± 1	7 ± 1	5 ± 0	4 ± 0	8 ± 1	6 ± 1	9 ± 1	10 ± 1	7 ± 1	10 ± 1	12 ± 1
	J	14 ± 1	29 ± 1	28 ± 1	30 ± 1	23 ± 1	22 ± 1	23 ± 1	23 ± 1	18 ± 1	33 ± 1	22 ± 1	36 ± 1	37 ± 1	40 ± 1	25 ± 1	26 ± 1	17 ± 1
	p	58 ± 1	41 ± 1	73 ± 1	82 ± 1	37 ± 1	45 ± 1	35 ± 1	29 ± 1	30 ± 1	55 ± 1	131 ± 1	69 ± 1	64 ± 1	84 ± 1	63 ± 1	75 ± 1	121 ± 1
Qga1	F	268 ± 34	199 ± 7	230 ± 8	89 ± 11	119 ± 12	94 ± 6	48 ± 1	63 ± 1	40 ±4	426 ± 36	185 ± 7	68 ± 1	238 ± 10	108 ± 4	53 ± 5	91 ± 1	232 ± 1
	J	199 ± 6	163 ± 18	202 ± 8	87 ± 5	89 ± 4	51 ± 1	45 ± 1	31 ± 1	23 ± 1	357 ± 26	129 ± 15	46 ± 1	207 ± 9	75 ± 1	47 ± 1	85 ± 1	63 ± 1
	p	338 ± 6	627 ± 18	270 ± 8	138 ± 5	187 ± 9	133 ±1	111 ± 3	69 ± 1	64 ± 3	619 ± 4	345 ± 2	708 ± 1	361 ± 8	129 ± 1	72 ± 4	155 ± 4	741 ±1
Qglu	F	289 ± 13	222±	200 ± 7	115 ± 9	188 ± 23	78 ± 3	28 ± 1	35 ± 3	34 ± 3	27,838	62 ± 3	37 ± 1	617 ± 17	53 ± 3	20 ± 1	74 ± 1	143 ± 3
	J	226 ± 17	191 ± 20	200 ± 5	125 ± 0	146 ± 1	47 ± 0	25 ± 1	18 ± 2	20 ± 9	231 ± 1	46 ± 1	27 ± 4	132 ± 1	34 ± 3	17 ± 4	74 ± 2	43 ± 1
	p	344 ± 15	277 ± 37	240 ± 21	183 ± 44	300 ± 13	115 ±7	61 ± 4	41 ± 1	55 ± 9	403 ± 18	12,418	42 ± 0	241 ± 5	63 ± 6	33 ± 1	125 ± 25	431 ± 71
Qrut	F	246 ± 27	240 ± 14	166 ± 5	224 ± 15	242 ± 15	91 ± 1	82 ± 1	122 ± 7	95 ± 7	0	195 ± 13	115 ± 1	242 ± 6	165 ± 5	23 ± 0	84 ± 1	73 ± 1
	J	232 ± 6	245 ± 13	164 ± 3	229 ± 10	205 ± 5	60 ± 2	57 ± 2	47 ± 2	50 ± 2	0	135 ± 6	64 ± 1	214 ± 2	84 ± 2	19 ± 1	83 ± 2	18 ± 2
	p	819 ± 10	225 ± 8	118 ± 3	202 ± 7	255 ± 20	70 ± 1	124 ± 5	73 ± 4	84 ± 4	1 ± 0	221 ± 6	61 ± 3	25,715	100 ± 5	21 ± 1	74 ± 1	221 ± 1
Ka	F	7 ± 1	5 ± 0	5 ± 0	6 ± 1	6 ± 1	6 ± 1	2 ± 0	4 ± 0	30	15 ± 1	2 ± 0	3 ± 0	8 ± 1	5 ± 0	1 ± 0	61	9 ± 1
	J	50	60	6 ± 0	70	5 ± 0	3 ± 0	2 ± 0	2 ± 0	2 ± 0	12 ± 0	1 ± 0	2 ± 0	6 ± 0	3 ± 0	1 ± 0	4 ± 0	2 ± 0
	p	3 ± 0	3 ± 0	4 ± 0	7 ± 0	2 ± 0	5 ± 0	4 ± 0	4 ± 0	3 ± 0	4 ± 0	7 ± 0	9 ± 0	4 ± 0	10 ± 0	4 ± 0	7 ± 0	10 ± 0
Isor	p	7 ± 0	7 ± 0	5 ± 0	21 ± 0	5 ± 0	1 ± 0	1 ± 0	0	0	0	2 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
PCA	p	46 ± 6	27 ± 4	40 ± 3	128 ± 10	8 ± 0	4 ± 0	4 ± 0	5 ± 0	5 ± 0	4 ± 0	17 ± 0	11 ± 0	4 ± 0	15 ± 0	18 ± 2	13 ± 1	17 ± 0
Caf	p	24 ± 1	18 ± 2	24 ± 3	67 ± 7	13 ± 1	16 ± 1	20 ± 1	23 ± 3	21 ± 1	21 ± 2	34 ± 1	51 ± 2	17 ± 1	51 ± 2	44 ± 2	34 ± 2	38 ± 2
P_B1	F	8 ± 1	10 ± 1	16 ± 1	40 ± 4	22 ± 1	26 ± 1	24 ± 5	28 ± 5	40 ± 2	26 ± 5	6 ± 1	31 ± 1	30 ± 1	32 ± 1	12 ± 1	21 ± 1	22 ± 1
	J	3 ± 0	5 ± 0	8 ± 1	18 ± 1	10 ± 1	14 ± 1	16 ± 1	18 ± 1	21 ± 1	19 ± 1	4 ± 0	18±	22 ± 1	17 ± 1	6 ± 0	14 ± 1	10 ± 1
	p	24 ± 1	25 ± 2	42 ± 4	75 ± 3	51 ± 2	72 ± 3	49 ± 2	60 ± 1	98 ± 9	99 ± 7	24 ± 3	68 ± 7	100 ± 10	61 ± 4	32 ± 3	68 ± 5	57 ± 1
P_B2	F	79 ± 1	94 ± 0	4 ± 7	80 ± 8	66 ± 8	49 ± 4	75 ± 1	97 ± 1	78 ± 1	60 ± 1	43 ± 1	118 ± 1	36 ± 1	107 ± 2	42 ± 5	44 ± 1	92 ± 1
	J	28 ± 5	40 ± 7	49±	42 ± 1	33 ± 5	23 ± 6	47 ± 1	71 ± 2	36 ± 1	42 ± 3	24 ± 1	66 ± 2	28 ± 1	62 ± 3	25 ± 4	29 ± 3	33±
	p	162 ± 7	172 ± 7	186 ± 7	185 ± 10	176 ± 16	150 ±14	188 ± 13	259 ± 6	209 ± 12	255 ± 18	174 ± 10	293 ± 7	139 ± 22	271 ± 19	145 ±4	164 ± 3	306 ± 1
P_C1	F	93 ± 1	105 ± 1	88 ± 1	96 ± 1	78 ± 1	53 ± 1	77 ± 1	100 ± 1	83 ± 1	70 ± 1	40 ± 1	142 ± 1	49 ± 1	124 ± 1	29 ± 2	55 ± 1	103 ± 2
	J	34 ± 1	44 ± 10	53 ± 11	38 ± 11	36 ± 12	26 ± 0	41 ± 1	66 ± 1	43 ± 1	41 ± 1	26 ± 10	69 ± 12	26 ± 13	67 ± 11	20 ± 11	40 ± 12	41 ± 11
	p	187 ± 24	201 ± 7	222 ± 24	234 ± 11	202 ± 18	163 ± 3	187 ± 14	267 ± 32	240 ± 25	273 ± 5	189 ± 9	308 ± 44	188 ± 0	282 ± 24	150 ± 5	185 ± 4	312 ± 23

NCha—neochlorogenic acid; ChA—chlorogenic acid; Cy_gal—cyanidin-3-galactoside; Cy.glu—cyanidin-3-O-glucoside; Cy_ara—cyanidin-3-arabinoside; Ecat—epicatechin, Cat—catechin, Coum—p-coumaric acid, Caf—caffeic acid, Fer—ferulic acid, Ka—kaempferol, Isor—isorhamnetin, Q—quercetin, Qgal—quercetin-3-O-galactoside; Qglu—quercetin 3-glucoside; Qrut—quercetin-3-O-rutinoside; and P_B1, P_B2, and P_C1—procyanidins B1, B2, and C1.



(**a**) (**b**) (**c**)

Figure 4. PCA score plots of different Sorbus fruit (**a**), juice (**b**), and pomace (**c**) samples.

3.4. Correlation Analysis

The correlation analysis demonstrated the significant correlations between the ORAC, ABTS$^{\bullet+}$, and DPPH$^{\bullet}$ scavenging values and the main phenolic groups in the *Sorbus* fruit, juice, and pomace fractions. As presented in Table 4, relatively strong positive correlations were found between all antioxidant assays using the pomace, fruit, and juice extracts and their TPC ($0.49 < R^2 < 0.95$) and ACY contents ($0.48 < i^2 < 0.89$). The correlations between ORAC, ABTS$^{\bullet+}$, and DPPH$^{\bullet}$ scavenging values and FLAVO contents of three extracts was moderate ($0.47 < R^2 > 0.66$), except the correlation between DPPH$^{\bullet}$ and FLAVO of fruit, which was weak ($R^2 = 0.28$).

Table 4. Correlation coefficients (R^2) between the content of different groups of polyphenolic compounds and the antioxidant capacity of 16 *Sorbus* fruit, juice, and pomace extracts.

	TPC			**HCA**			**ACY**			**FLAVO**		
Part	F	J	P	F	J	P	F	J	P	F	J	P
ABTS	0.872	0.723	0.749	0.537	0.558	0.105	0.751	0.751	0.820	0.658	0.591	0.491
DPPH	0.547	0.948	0.810	0.221	0.616	0.188	0.527	0.893	0.886	0.278	0.658	0.514
ORAC	0.822	0.493	0.708	0.512	0.265	0.289	0.685	0.476	0.517	0.652	0.567	0.466

TPC—total phenolic content, HCA—hydroxycinnamic acids, ACY—anthocyanins, FLAVO—flavanols, F—fruit, J—juice, P—pomace.

There was no correlation found between radical scavenging values and the contents of FLAVA in fruit, juice, and pomace extracts.

In the case of pomace extracts, the weak correlations were found between the radical scavenging values determined by ORAC, ABTS$^{\bullet+}$, and DPPH$^{\bullet}$ methods and the contents of HCA; however, there was a moderate correlation between the ORAC and ABTS$^{\bullet+}$ scavenging values and HCA content in the fruit, as well as between ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ scavenging values and HCA content in juice extracts. The differences in correlations with polyphenolic groups and various radical scavenging methods, while using the same extracts, can be explained by the different reaction mechanisms in ORAC, ABTS$^{\bullet+}$, and DPPH$^{\bullet}$ assays, as described earlier (see Section 3.2).

The correlations between antioxidant assays and phenolic groups are different while using the whole fruit, pressed juice, or pomace for the analysis. In the current study working with 16 *Sorbus* cultivars and wild rowanberry, the major part (on average 85%) of the weight of fresh rowanberries comprised juice; therefore, it is expected that the fruit

and juice could have comparable composition. The correlation analysis demonstrated comparable correlations between the antioxidant assays and polyphenolic groups of fruit and juice. The antioxidant activity of pomace samples, which consist mainly of peel and seeds, is influenced by TPC and ACY contents and moderately by FLAVO content in the samples, while in the case of the fruit and juice samples, HCA contents have an additional effect on radical scavenging values. Compared to the fruit and juice extracts, pomace extracts hold higher concentrations of protocatechuic acid and isorhamnetin, but also epicatechin, catechin, and procyanidins B1, B2, and C1, making the pomace fraction a considerable source of natural antioxidants.

4. Conclusions and Further Perspectives

The high yield and good antioxidant potential of starting materials were essential while selecting the potential cvs for total valorization. Therefore, the goal of the current study was the antioxidants characterization of the fruit, juice, and pomace of 16 best yielding sweet rowanberry (*Sorbus aucuparia* L.) cultivars and wild rowanberry grown in Estonia. Although 9 of 16 selected cvs and wild rowanberry were previously analyzed for polyphenolic content and antioxidant activity by different authors, it was relevant to compare the antioxidant characteristics of these best yielding cvs grown in different climatic conditions. Moreover, according to our knowledge the cultivar-based pomace characterizations have never been conducted.

In our study, twenty different phenolic compounds were detected in the acidified ethanolic extracts of cultivated and wild sweet rowanberry cultivars by UHPLC-MS. The contents of individual phenolic compounds in every investigated *S. aucuparia* L. cultivar, as well as the composition of rowanberry fruit, juice, and pomace samples, differed significantly from each other. In addition, the different constituents in the tested samples influenced the anti-radical scavenging activity in different fruit fractions of cultivars. Although the fruit and juice samples contain more ACYs than the pomace samples, the antioxidant characteristics of both are influenced by this group of polyphenols. On the other hand, the pomace samples, where the hydroxycinnamic acids dominated, were not affected by these components, and vice versa, the fruit and juice samples with lower HCA contents were more influenced by these polyphenolic acids. The fruit and juice samples of the sweet rowanberry hybrids Likernaja and Burka, crossbreeds with *Aronia melanocarpa* (Michx.) and *Aronia arbutifolia* L., respectively, had the highest contents of ACYs and HCAs. The pomace samples of the mentioned hybrids also had higher contents of ACYs when compared to the other investigated cultivars. As a significant part of phytochemicals remain in the rowanberry pomace fraction, it can be a potential source of functional ingredients for the biorefining process to increase the utilization of sweet rowanberry cultivars.

Author Contributions: V.S. experimental works, designing of the experiments and methodology, writing—original draft and editing; P.R.V. methodology, writing—review and editing, validation, supervision; R.B. methodology, editing, supervision; R.R. methodology, editing, supervision; R.K. methodology, investigation; A.A. methodology. All authors have read and agreed to the published version of the manuscript.

Funding: The theme of this research article is based on the ongoing project VALORTECH, which has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 810630. In addition, funding was received from Mobilitas Pluss ERA-Chair support (Grant No. MOBEC006 ERA Chair for Food (By-) Products Valorisation Technologies of the Estonian University of Life Sciences).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in article.

Acknowledgments: This work is supported by Plantvalor Project "PlantValor–full-scale product development service in synergy with the traditional activities of Polli Horticultural Research Centre 2020–2023", which has received funding from the European Regional Development Fund; and by ERA Chair in VALORTECH project, which has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 810630. The authors also acknowledge Estonian Ministry of Rural Affairs for the programmes "Collection and Conservation of Plant Genetic Resources for Food and Agriculture in 2014–2020".

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Polyphenols Market Size Worth $2.08 Billion By 2025_CAGR_7.2%. *Gd. View Res. Inc. Electron.* 2019. Available online: https://www.grandviewresearch.com/press-release/global-polyphenols-market (accessed on 5 November 2021).
2. Di Lorenzo, C.; Colombo, F.; Biella, S.; Stockley, C.; Restani, P. Polyphenols and human health: The role of bioavailability. *Nutrients* **2021**, *13*, 273. [CrossRef]
3. Sarv, V.; Venskutonis, P.R.; Bhat, R. The sorbus spp.—underutilised plants for foods and nutraceuticals: Review on polyphenolic phytochemicals and antioxidant potential. *Antioxidants* **2020**, *9*, 813. [CrossRef] [PubMed]
4. Venskutonis, P.R. Berries. In *Valorization of Fruit Processing By-Products*, 1st ed.; Galanaskis, C.M., Ed.; Academic Press: London, UK, 2020; pp. 95–125.
5. De Ancos, B.; Colina-Coca, C.; González-Peña, D.; Sánchez-Moreno, C. Bioactive compounds from vegetable and fruit by-products. In *Biotechnology of Bioactive Compounds: Sources and Applications*; John Wiley & Sons: Hoboken, NJ, USA, 2015; pp. 1–34.
6. Heinonen, M. Antioxidant activity and antimicrobial effect of berry phenolics–a Finnish perspective. *Mol. Nutr. Food Res.* **2007**, *51*, 684–691. [CrossRef] [PubMed]
7. Shikov, A.N.; Pozharitskaya, O.N.; Makarov, V.G.; Wagner, H.; Verpoorte, R.; Heinrich, M. Medicinal Plants of the Russian Pharmacopoeia; Their history and applications. *J. Ethnopharmacol.* **2014**, *154*, 481–536. [CrossRef]
8. Mrkonjić, Z.O.; Nađpal, J.D.; Beara, I.N.; Sabo, V.S.A.; Četojević-Simin, D.D.; Mimica-Dukić, N.M.; Lesjak, M.M. Phenolic profiling and bioactivities of fresh fruits and jam of Sorbus species. *J. Serbian Chem. Soc.* **2017**, *82*, 651–664. [CrossRef]
9. Berna, E.; Kampuse, S.; Straumite, E. The suitability of different rowanberry cultivars for production of fruit marmalade. In Proceedings of the Annual 18th International Scientific Conference "Research for Rural Development", Jelgava, Latvia, 16–18 May 2012; Treija, S., Skuja, I., Eds.; Latvia University of Agriculture: Jelgava, Latvia, 2012; Volume 1, pp. 109–116.
10. Sokolov, V.V.; Savel'ev, N.I.; Goncharov, N.P.I.V. Michurin'S work on expansion of the plant horticulture assortment and improvement of food quality. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact, Appl. Sci.* **2015**, *69*, 190–197. [CrossRef]
11. Mlcek, J.; Rop, O.; Jurikova, T.; Sochor, J.; Fisera, M.; Balla, S.; Baron, M.; Hrabe, J. Bioactive compounds in sweet rowanberry fruits of interspecific Rowan crosses. *Cent. Eur. J. Biol.* **2014**, *9*, 1078–1086. [CrossRef]
12. Rengarten, G.A.; Sorokopudov, V.N. Introduction and selection of Sorbus as a food plant in countries of the world. *Ekosistemy* **2019**, *18*, 89–96.
13. Kylli, P.; Nohynek, L.; Puupponen-Pimiä, R.; Westerlund-Wikström, B.; McDougall, G.; Stewart, D.; Heinonen, M. Rowanberry phenolics: Compositional analysis and bioactivities. *J. Agric. Food Chem.* **2010**, *58*, 11985–11992. [CrossRef]
14. Hukkanen, A.T.; Pölönen, S.S.; Kärenlampi, S.O.; Kokko, H.I. Antioxidant capacity and phenolic content of sweet rowanberries. *J. Agric. Food Chem.* **2006**, *54*, 112–119. [CrossRef]
15. Zymone, K.; Raudone, L.; Raudonis, R.; Marksa, M.; Ivanauskas, L.; Janulis, V. Phytochemical profiling of fruit powders of twenty *Sorbus* L. Cultivars. *Molecules* **2018**, *23*, 2593. [CrossRef] [PubMed]
16. Jurikova, T.; Sochor, J.; Mlcek, J.; Balla, S.; Klejdus, B.; Baron, M.; Ercisli, S.; Ozturk Yilmaz, S. Polyphenolic profile of interspecific crosses of rowan (*Sorbus aucuparia* L.). *Ital. J. Food Sci.* **2014**, *26*, 317–324.
17. Sarapuu, H.; Arus, L.; Rätsep, R. Physical parameters and biochemical composition of fruits in different rowan tree (Sorbus sp.) cultivars and hybrids. *Molecules* **2018**, *23*, 2593.
18. Serpen, A.; Capuano, E.; Fogliano, V.; Gökmen, V. A new procedure to measure the antioxidant activity of insoluble food components. *J. Agric. Food Chem.* **2007**, *55*, 7676–7681. [CrossRef] [PubMed]
19. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
20. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
21. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
22. Prior, R.L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *J. Agric. Food Chem.* **2003**, *51*, 3273–3279. [CrossRef]
23. Dávalos, A.; Gómez-Cordovés, C.; Bartolomé, B. Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *J. Agric. Food Chem.* **2004**, *52*, 48–54. [CrossRef]

24. Bobinaitė, R.; Kraujalis, P.; Tamkutė, L.; Urbonavičienė, D.; Viškelis, P.; Venskutonis, P.R. Recovery of bioactive substances from rowanberry pomace by consecutive extraction with supercritical carbon dioxide and pressurized solvents. *J. Ind. Eng. Chem.* **2020**, *85*, 152–160. [CrossRef]
25. Apak, R.; Özyürek, M.; Güçlü, K.; Çapanoğlu, E. Antioxidant activity/capacity measurement. 2. Hydrogen atom transfer (HAT)-based, mixed-mode (electron transfer (ET)/HAT), and lipid peroxidation assays. *J. Agric. Food Chem.* **2016**, *64*, 1028–1045. [CrossRef] [PubMed]
26. Huang, D.; Boxin, O.U.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856. [CrossRef] [PubMed]
27. Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302. [CrossRef]
28. Kampuss, K.; Kampuse, S.; Berņa, E.; Krūma, Z.; Krasnova, I.; Drudze, I. Biochemical composition and antiradical activity of rowanberry (*Sorbus* L.) cultivars and hybrids with different Rosaceae L. cultivars. *Eur. J. Hortic. Sci.* **2009**, *59*, 195–201.
29. Bobinaitė, R.; Grootaert, C.; Van Camp, J.; Šarkinas, A.; Liaudanskas, M.; Žvikas, V.; Viškelis, P.; Rimantas Venskutonis, P. Chemical composition, antioxidant, antimicrobial and antiproliferative activities of the extracts isolated from the pomace of rowanberry (*Sorbus aucuparia* L.). *Food Res. Int.* **2020**, *136*, 109310. [CrossRef] [PubMed]
30. Mikulic-Petkovsek, M.; Krska, B.; Kiprovski, B.; Veberic, R. Bioactive components and antioxidant capacity of fruits from nine Sorbus genotypes. *J. Food Sci.* **2017**, *82*, 647–658. [CrossRef]

III

Sarv, V., Kerner, K., Venskutonis, P.R., Rocchetti, G., Pier Paolo Becchi, P.P., Lucini, L., Tänavots, A., Bhat, R. 2023. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs Food Chemistry: X, 19, 100761. doi. org/10.1016/j.fochx.2023.100761

Food Chemistry: X 19 (2023) 100761

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs

Viive Sarv [a,b,*], Kristi Kerner [b,c,g], Petras Rimantas Venskutonis [a,c], Gabriele Rocchetti [d], Pier Paolo Becchi [e], Luigi Lucini [e], Alo Tänavots [f,g], Rajeev Bhat [b]

[a] Polli Horticultural Research Centre, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Uus 2, Polli, Mulgi Parish, 69108 Viljandi, Estonia
[b] ERA Chair for Food (By-) Products Valorisation Technologies (VALORTECH), Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006 Tartu, Estonia
[c] Department of Food Science and Technology, Kaunas University of Technology, Radvilėnų pl. 19, LT-50254 Kaunas, Lithuania
[d] Department of Animal Science, Food and Nutrition (DiANA), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy
[e] Department for Sustainable Food Process (DiSTAS), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy
[f] Chair of Animal Breeding and Biotechnology, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 62, 51006 Tartu, Estonia
[g] Chair of Food Science and Technology, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 56/5, 51006 Tartu, Estonia

ARTICLE INFO

Keywords:
Rowanberry pomace
Food metabolomics
Lipid oxidation
Antioxidants
Meat quality

ABSTRACT

In this study, a rowanberry pomace defatted with supercritical CO_2 (2%-AC), its ethanolic extract (1%-E) and extraction residue (2%-R), were tested in meatball preparation. The meatballs with 1%-E demonstrated the highest in vitro radical scavenging capacity. In the case of 1%-E the pH of meatballs was significantly lower compared to the control sample ($P = 0.0132$) on the 5-day. The lowest cooking loss was achieved when the meatballs contained mainly fibre-rich 2%-R. The UHPLC method detected 184 metabolites, including strong antioxidants, such as chlorogenic acids, 3′,4′-methylenedioxy-5,7-dimethylepicatechin, hyperin, isoquercitrin. The 1%-E was particularly effective against the development of unpleasant off-flavours caused by carbonyl compounds. Consistently, the decrease in lipid oxidation, indicated by reduced 7-dodecenal and 2,4-heptadienal contents, has been observed following the addition of rowanberry extract to meatballs. Metabolomics coupled with conventional quality evaluations provided a deeper understanding of the potential utilization and valorisation of different rowanberry pomace extracts as meat ingredients.

1. Introduction

Meat products are significantly susceptible to the decline of quality caused by oxidative processes, especially considering processing and shelf life. The loss of quality in meat, usually supported by lipid oxidation and the formation of undesired compounds, affects meat composition and processing characteristics (Munekata et al., 2020). The hydroperoxides formed while cooking meat may decompose and form volatile organic compounds such as aldehydes, alkanes, alkenes, ketones, alcohols, esters and acids (Domínguez et al., 2019). These compounds are responsible for the deterioration of the colour, texture and flavour of meat-derived products and the loss of pigments and vitamins in meat products (Domínguez et al., 2019; Aminzare et al., 2019). Grinding and heating disarrange the muscle cell configuration, deactivating antioxidative enzymes and initiating non-heme iron, increasing lipid oxidation (Gallego et al., 2015). Therefore, it is challenging food scientists to find ways to inhibit or delay these reactions by utilizing various antioxidant compounds. Whereas synthetic preservatives were mainly used in previous years, recently the emphasis has shifted to safer natural antioxidants (Domínguez et al., 2019).

Recently, food metabolomics or food omics, which aims to analyse small molecules (metabolites), such as pathogens in food systems, has

* Corresponding author at: Polli Horticultural Research Centre, Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Uus 2, Polli, Mulgi Parish, 69108 Viljandi, Estonia.
E-mail addresses: viive.sarv@emu.ee (V. Sarv), kristi.kerner@emu.ee (K. Kerner), rimas.venskutonis@ktu.lt (P. Rimantas Venskutonis), gabriele.rocchetti@unicatt.it (G. Rocchetti), pierpaolo.becchi@unicatt.it (P. Paolo Becchi), luigi.lucini@unicatt.it (L. Lucini), alo.tanavots@emu.ee (A. Tänavots), rajeev.bhat@emu.ee (R. Bhat).

https://doi.org/10.1016/j.fochx.2023.100761
Received 28 December 2022; Received in revised form 5 June 2023; Accepted 16 June 2023
Available online 28 June 2023

gained attention (Fiehn, 2002). Novel analytical instruments, as well as metabolite databases, enable analysing thousands of metabolites in a single analysis and identifying novel metabolites present in food (Beale et al., 2016). The non-targeted approaches allow identifying unknown compounds relevant to food systems and identifying the biomarkers of spoilage (Rocchetti, Bernardo, et al., 2020).

In order to avoid the deteriorative impact of reactive oxygen species (ROS) on meat products, different plant-based ingredients possessing antioxidant capacity, such as the extracts obtained from fruits, vegetables, herbs, and spices, have been tested as meat ingredients (Aminzare et al., 2019). Various berries, such as blueberries, blackberries, cranberries, and grapes, have demonstrated their effectiveness as ingredients possessing antioxidant capacity for stabilizing meat products due to their high contents of antioxidant polyphenols (Lorenzo et al., 2018). Moreover, berry pomace, the solid residue of juice production (Tamkute et al., 2021) has shown good antioxidant potential. Comparing the antioxidant capacities of different berry pomace extracts, Babaoğlu et al. (Babaoğlu et al., 2022) found that the red currant pomace extract showed the highest value in the DPPH● assay, while the water extract of chokeberry pomace exhibited the highest flavonoid and total phenolic content (TPC). In the case of incorporating the water extract of chokeberry pomace into meat products, beef patties' oxidative stability and microbiological acceptability increased during storage in the refrigerator (Babaoğlu et al., 2022). Kähkönen et al. found that 0.05 % rowanberry extract inhibited over 90% of the formation of methyl linoleate-conjugated diene hydroperoxides (Kähkönen et al., 1999). Moreover, rowanberry jam has been considered a suitable ingredient for meat dishes (Hallmann et al., 2011). Compared to the rowanberry juice or fruit samples, pomace samples have demonstrated even higher average TPCs (Sarv et al., 2021). The rowanberry pomace samples, especially originated from the hybrid cultivars (cvs), but also from some other selected rowanberry varieties possessed significant antioxidant capacity values (Sarv et al., 2021). To the best of our knowledge, only one article is available on the use of rowanberry extract in meat products; wherein methanol and ethanol extracts of the whole berries were tested in the emulsified raw pork burger patties, while pomace ingredients have not been tested in meat previously (Ganhão et al., 2010). Considering the tendency of upcycling agro-food processing by-products into higher benefit products it was of interest to test such ingredients in meat, In general, the reports on the use of processed by consecutive extractions berry pomace products in foods are rather scarce.

In terms of their antioxidant properties, it was hypothesized that the rowanberry pomace based ingredients inhibit lipid oxidation in meatballs during meat processing and/or storage. The untargeted metabolomic approach was used for evaluating the changes of metabolites in meatballs with the addition of rowanberry pomace-based ingredients possessing antioxidant capacity during their storage time period.

2. Materials and methods

2.1. Plant material and extracts preparation

Sweet rowanberry cvs Likernaja (the hybrid of *S. aucuparia* × *Aronia melanocarpa*), Solnechnaja (the seedling of *S.aucuparia*) and wild rowanberry were harvested in autumn 2021 from Polli Experimental Station, (South Estonia, 58°07̇44.5̇N, 25°32̇16.8̇E). All fruits were immediately frozen and stored at −20 °C. The fruit was defrosted before extracting the juice by a low-speed juicer Smeg SJF01CREU (Smeg S.p. A, Guastalla, Italy). The pomace parts, which accounted for approximately 15–20% of the weight of the fresh rowanberries (Sarv et al., 2021), were freeze-dried in a VirTis Advantage Plus Benchtop Freeze Dryer Model XL-70 (SP Industries, Warminster, PA, USA) for 72 h at 30 μbar. Subsequently, three pomace samples were mixed and ground in Retsch cutting mill Retsch SM 300, (Retsch GmbH, Haan, Germany) with sieve holes diameter of 5 mm to obtain a homogenous batch. This sample was defatted by extracting with supercritical CO_2 in SCF extraction equipment Separex 5 (Champigneulles, France) for removing lipophilic substances at 40 MPa pressure, 40∘C temperature and 150 min extraction time (Tamkute et al., 2021) to obtain the 1st ingredient (AC) for meatballs. The lipophilic CO_2 extract was not used in the meat tests due to the remarkable content of polyunsaturated fatty acids in berry seeds, which may accelerate the formation of oxidation products in meatballs during storage (Venskutonis, 2020).

AC was further extracted with 1:1 (v/v) ethanol/water at solid/liquid ratio of 1:10 (w/v) using microwave-assisted extraction (MAE) for 15 min at power 300 W. After the extraction, the extract was filtered. The EtOH part of the supernatant was dried in a rotary evaporator, and the remaining water was freeze-dried in a VirTis Advantage Plus Benchtop Freeze Dryer Model XL-70 (SP Industries, Warminster, PA, USA). The dried extract was stored in a sealed package in a freezer (-20 °C) as the 2nd ingredient (E) for meatballs. The extraction residue was freeze-dried and stored as the 3rd ingredient (R) in the grip seal polythene bag at room temperature.

2.2. Preparation of meatballs

The meatballs were prepared according to the protocol of Kerner et al. (Kerner et al., 2021) with modifications. Briefly, the minced pork and salt were purchased from the local (Tartu, Estonia) butcher's shop and the food store, respectively. The components were mixed according to the recipe and the raw mixture was divided into the following portions: the control sample (88% of minced pork, 11% water, 1% salt), and the samples with ingredients AC, R and E, each with the concentrations of 1%, 2%, 3% and 5%. Six voids (Ø 4.5 cm, depth 2 cm) in the self-made moulds were filled with the raw meatball mixture. After weighing, the meatballs were cooked at 145 °C in the oven Inoxtrend E1CUA-107E (Santa Lucia di Piave, Italy) for 15 min. The cooked meatballs were cooled down to room temperature and weighed and packed into a Vision Pack Srl VP01 (Packaging Factory Holding, Lallio, Italy) under a modified atmosphere consisting of 70 % N_2 and 30% CO_2 provided by Linde GAS Limited Company (Tallinn, Estonia). To understand the effect of rowan ingredients on the physicochemical parameters of pork meatballs during cold storage, the time points 0 and 5 were chosen according to USDA Food Safety and Inspection Service https://www.fsis.usda.gov/ . Accordingly, the ideal shelf-life period of properly stored, cooked meatballs without artificial preservatives is 3–4 days in the refrigerator. Therefore, packed meatballs were stored at + 4 °C and analyzed at 0 and 5 days of storage.

2.3. Sensory evaluation

The sensory assessment of cooked meatballs was conducted by nine randomly selected trained assessors from the Estonian University of Life Sciences, Chair of Food Science and Technology, in a specially designed room with individual booths. The fresh meatballs were warmed to 55–70 °C in a microwave oven (Moulinex Micro-Chef V98, Ecully, France) and cut in half before sensory assessment. The sensory attributes for the valuation of cooked meatballs were the odour, appearance, colour, taste, juiciness, and texture. The widely used hedonic 9-point scale (Wichchukit & O'Mahony, 2015), where the points 9; 5 and 1 indicate very good, satisfying, and not satisfying assessment, respectively, was applied for sensory evaluation.

2.4. Determination of quality characteristics

The cooking loss of meatballs was calculated as the weight difference between raw and cooked but cooled to the room temperature samples in percentages. Prior to the chemical analyses, such as fat (EVS-ISO 2446:2001, Gerber method), moisture (EVS-ISO 1442:1999), protein (EVS-ISO 937:1978, Kjeldahl method), and ash content (ISO 936:1999), the meatball samples were homogenised using the Retsch GM200 laboratory homogeniser (Retsch Gmbh & Co, Haan, Germany). Seven

2Go™ pH-meter (Mettler-Toledo AG Analytical, Schwerzenbach, Switzerland) was used to determine the pH of meatball samples (5 g) homogenised with 50 mL of 0.1 M potassium chloride solution. The water activity analyser (Aqua Lab, Model Series 3 TE, Decagon Devices, Inc., Washington, DC, USA) was used to determine the water activity (aw) by achieving the equilibrium humidity of air in a tightly closed chamber. The X-Rite 964 spectrophotometer (X-Rite, Grand Rapids, MI, USA) was used for taking three replicate colour measurements of each freshly cut meatball sample from different places. The measurements were expressed numerically by CIE (International Commission on Illumination) Lab system values, where L^*, a^*, b^* mark the lightness, redness, yellowness, respectively (Mokrzycki & Tatol, 2012).

2.5. Determination of total phenolic content and in vitro radical scavenging activity

Two in vitro spectrophotometric analyses, the total phenolic content (TPC) and 2.2-diphenyl-picrylhydrazyl (DPPH•) scavenging assay, were used for preliminary screening of meat ingredients AC, R and E. The (TPC) was measured according to the method of Folin–Ciocalteu (FC) (Folin & Ciocalteu, 1927) with modifications using the gallic acid (GA) standards. The free radical scavenging capacity assay (DPPH•) with slight modifications was, according to Brand-Williams et al. (Brand-Williams et al., 1995) using Trolox as a positive control. The absorbance values of the samples during the TPC and DPPH• assays were measured at 760 nm and 515 nm, using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Both spectrophotometric assays were performed in four replicates using laboratory grade chemicals purchased from Sigma-Aldrich (Steinheim, Germany).

2.6. Untargeted profiling by UHPLC-HRMS of the different meatballs

The untargeted metabolomics was used to evaluate storage time effects on prepared meatballs and the phytochemical profile of the 3 different rowanberry extracts. The time points for observation of the changes in metabolomics were selected as the day of preparation- day 0, the longest ideal period for storing homemade meatballs – day 4, and to study the possible oxidation process of packed meatballs-day 14.

Therefore, the lyophilized pork meatballs were extracted following the protocol previously reported by Pateiro et al. (Pateiro et al., 2018), with minor modifications. Briefly, one gram of each sample was extracted with 10 mL of an 80% aqueous methanol (v/v) solution (both LC-MS grade, VWR, Milan, Italy) added with 0.1% (v/v) formic acid. This mixture was subjected to an extraction system through Ultra-turrax (Ika T10, Staufen, Germany) for 5 min at room temperature. The corresponding extracts were centrifuged (Eppendorf 5810R, Hamburg, Germany) at 7800 × *g* for 15 min at 4 °C and then filtered using 0.22 μm cellulose syringe filters. Finally, the filtered samples were transferred to amber vials until instrumental analysis.

In this work, the untargeted profiling analysis was done using high-resolution mass spectrometry (HRMS) based on a Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) coupled to a Vanquish ultra-high-pressure liquid chromatography (UHPLC) pump and equipped with heated electrospray ionization (HESI)-II probe (Thermo Scientific, USA). Shortly, the chromatographic separation was carried out under a gradient of acetonitrile in water (from 6% to 94% in 35 min) as mobile phase, with 0.1% formic acid as a phase modifier, using BEH C18 (2.1x100 mm, 1.7 μm) analytical column maintained at 35 °C. The injection volume was 6 μL and elution was operated with a flow rate of 200 μL/min. Full scan MS analysis was performed under the positive ionization mode and with a nominal mass resolution of 70,000 FWHM at *m/z* 200. The injection sequence was randomized, with three replicates for each sample. Quality control (QC) samples (prepared by pooling same aliquots of each sample) were acquired in a data-dependent (TOP N = 3) MS/MS mode, and the Top N ions were selected for fragmentation under stepped (10, 20, 40 eV) Normalized Collisional Energy. The HESI parameters were previously optimized by Rocchetti et al. (Rocchetti et al., 2021).

The raw spectral data were processed using MS-DIAL software (version 4.80) (Tsugawa et al., 2015) for post-acquisition and data filtering procedures. The MS-DIAL parameters were adapted from previously published works on LC-MS untargeted metabolomics-based analysis (Rocchetti et al., 2021). The mass features were searched in the mass range of 80–1200 *m/z*, having a minimum peak height of 10,000 cps. Accurate mass tolerance for peak centroiding was 0.05 Da for MS and 0.1 Da for MS/MS analysis. Retention time information was excluded from the calculation of the total identification score. The MS and MS/MS tolerance for identification was set to 0.05 Da and 0.1 Da, respectively. The identification step was based on mass accuracy, isotopic pattern (i.e., isotopic distribution, space, and abundance) and spectral matching. The total identification cut-off score was set to 50%, retaining the most common HESI + adducts. Annotation of meat metabolites was achieved against the comprehensive database known as FooDB (https://foodb.ca/). Furthermore, the software MS-Finder (Tsugawa et al., 2016) was used for in-silico fragmentation of the not annotated mass compounds, using the FooDB and Lipid Maps libraries, thus working according to a level 2 of confidence in annotation (i.e., putatively annotated compounds and structural confirmation according to spectral matching) (Salek et al., 2013). Only the compounds having an in-silico prediction score higher than 5 were retained.

2.7. Statistical and multivariate data analysis

The mean values and standard deviations (SD) of total phenolic contents (TPC) and DPPH• radical scavenging capacity (RSC) results were calculated using MS Excel 2016 and one-way analysis of the variance (ANOVA) at p value < 0.05. The statistical package R 4.2.0 was applied for statistical analysis (Minato Nakazawa, 2022) of assessors panel sensory scores and sensory score results visualization. The Linear Mixed-Effects Model (GLMM) was used to study the effects of variants, the effect of three replications and the storage period on the pH, aw and colour characteristics of the samples as well as the measurements of cooking loss, moisture, and protein and ash contents on the day 0. The Emmeans (Searle et al., 1980) package was applied for the pairwise comparison of groups and the model-assessed results were presented as least-square means.

The multivariate statistical analyses dealing with metabolomics were done using two different softwares, Mass Profiler Professional (version B.12.06; from Agilent Technologies) and SIMCA (version 16; from Umetrics, Malmo, Sweden) for data processing and normalization and supervised modelling, respectively. In this regard, both unsupervised and supervised multivariate statistics were used based on hierarchical cluster analysis (HCA), Principal Component Analysis (PCA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA). The OPLS-DA models were built considering the storage time period (i.e., 0, 4, and 14 days) under investigation, also recording the model validation parameters (goodness-of-fit R^2Y) and goodness-of-prediction Q^2Y). The VIP (i.e., variables importance in projection) selection method was then used to list the most relevant meat metabolites in prediction, considering only VIP markers characterized by values higher than 1. Finally, a Fold-Change (FC) analysis was done to check the direction and the intensity of variation of the marker compounds highlighted by the VIP selection method.

3. Results and discussion

3.1. Sensory evaluation and in vitro antioxidant capacity of meatballs

Due to the harmfulness of synthetic ingredients, various natural preservatives have recently been tested to inhibit lipid oxidation and extend the shelf-life of foods (Aziz & Karboune, 2018). However, applying plant-based ingredients to food products as preservatives may

be limited due to the flavour characteristics (Dussault et al., 2014). The astringent taste of rowanberry is a major obstacle to its consumption. Therefore, it is essential to know the acceptable dose of this ingredient to achieve the sensory quality of meatballs. In our research, three rowanberry pomace powders of cvs Likernaja, Solnechnaja and wild rowanberry were pre-selected as the ones with the highest antioxidant capacity (Sarv et al., 2021). These powders were defatted and mixed to obtain the 1st ingredient (AC). The 2nd ingredient was EtOH/water microwave extract of defatted pomace (E) and the 3rd was the extraction residue (R). The TPC of these three ingredients were analyzed and the TPC value of E was almost fivefold compared to AC and 17 times higher than R (Fig. 1a).

The minced pork (moisture 67.43%, protein 18.49%, fat 13.85%, and ash 0.96%) was mixed with 11% water and 1% salt as well as the rowanberry pomace-based ingredient. The concentrations from 1% to 5% of ingredients were sensory evaluated and the best of each group were selected for further tests. The meatballs without any ingredients were evaluated in every test as a reference.

The panellists gave the highest average score for the colour (7.8) of the samples with 3%-AC, followed by the juiciness of the samples with 1%-R and the control, both with an average score of 7.5. In addition, the samples with 1%-R achieved the best score of 7.5 for the taste. The odour was most acceptable in the case of 1%-R. The samples with more than 1%-E scored<5 for taste and were not acceptable for further use. Both AC and R with concentrations 1–3% got the average scores of sensory attributes more than 5; therefore, 2%-AC and 2%-R as well as 1%-E were chosen for further experiments.

The in vitro antioxidant capacity of lyophilized meatballs was tested, using their ability to scavenge stable diphenyl-picrylhydrazyl radical DPPH• assay. Compared to the meatballs without any ingredients (control), the meatballs with ingredients had remarkably higher DPPH• assay values: with the addition of 1%-E, 2%-AC, and 2%-R, the antioxidant potential of meatballs was more than 15-, 10- and 5- fold higher, respectively (Fig. 1b). These results can be crucial in slowing oxidation and deleterious processes occurring during meat processing and/or storage.

3.2. Proximate composition and cooking losses of meatballs

Adding plant-based fibres to meat products allows producers to supply the food with better texture or moisture holding capacity and reduce the content of animal-based proteins and saturated fatty acids (Paglarini et al., 2022). In the current study, the plant-based ingredients accounted just for 1–2%; therefore, the moisture, ash and protein contents were not affected remarkably.

However, the fat content in meatballs decreased by adding the plant-based ingredients, especially the fibre-rich AC and R. In addition, as indicated by ANOVA (Table 1), the ingredient R affected the juiciness of meatballs by keeping more than 2% higher moisture content compared to the control sample and by reducing the cooking loss more than 13%. These results agree with the previous study, where a remarkable decrease in cooking loss was achieved with 3% sugarcane fibre addition to meatballs (Mena et al., 2020). In contrast to fibre-rich ingredients, the lyophilized extract E increased the cooking losses significantly.

Table 1
Proximate composition of cooked pork meatballs and cooking losses.

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

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

3.3. Determination of physicochemical parameters

The main quality characteristics, which play an important role in defining consumers' preferences, are the juiciness, colour, freshness and tenderness of meat products. These quality characteristics are affected by the physicochemical parameters, such as pH, water activity (aw) and colour (Tamkute et al., 2021). The decrease in pH can lead to an unacceptable taste in food but also provide an inhibitory effect against spoilage or pathogenic microorganisms (Barcenilla et al., 2022).

In current study, after 5 days of storage at 4 °C, the pH was significantly lower in the meatballs with 1%-E compared to the control sample ($P = 0.0132$) (Fig. 2 a). The pH reduction in meatballs can be explained by the higher concentration of chlorogenic acids present in E, compared to the fibre-rich ingredients AC and R (Sarv et al., 2021). However, in the case of the samples with AC and R, the pH remained stable during the 5-days of storage, due to some content of chlorogenic acid, while the pH of the control sample increased. Tamkute et al. found that the pH of cooked ham samples with chokeberry extract remained constant during a prolonged (36 days) storage period at 4 °C, while the pH of control sample increased (Tamkute et al., 2021). The "easily perishable" meat products aw greater than 0.95 and pH greater than 5.2 must be stored at or < 5 °C (Halagarda & Wójciak, 2022). The measured aw values of the meatballs in this study ranged within 0.974–0.987 (Fig. 2 b), while the highest reduction (1.3%) in aw values were achieved when the fibre-rich 2%-R was added to meatball pastry. Such a small decrease in aw doesn't have any significant influence on the self-life and storage conditions of

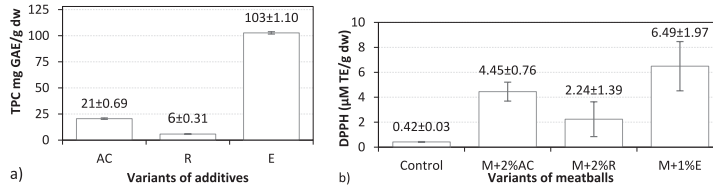


Fig. 1. The total phenolic content (TPC) of rowanberry pomace-based ingredients for meat a): Control—without ingredients, AC— defatted with supercritical CO_2 rowanberry pomace, E— EtOH/water extract of AC, R—extraction residue; and b) antioxidant capacity (DPPH) of meatballs with and without ingredients: Control—without ingredients, M + 2 %AC— meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace, M + 1 %E— meatballs with 1% of EtOH/water extract of AC, M + 2 %R— meatballs with 2% of extraction residue.

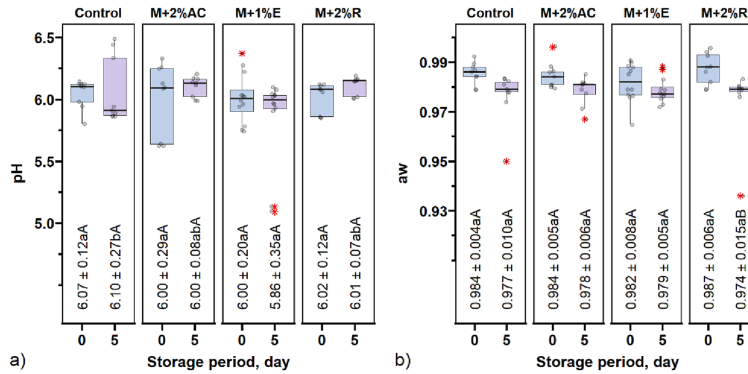


Fig. 2. Physicochemical parameters of meatballs (pH and aw) on the day of preparation and after five days of storage Actual values are presented with grey dots; outliers are denoted with a red asterisk; mean values are presented as least-square means ± standard deviations; different lowercase letters indicate the statistical difference ($p < 0.05$) between the variants within the same day; different capital letters indicate the statistical difference ($p < 0.05$) between the days within the same variant). Control—without ingredients, M + 2 %AC— meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace, M + 1 %E— meatballs with 1% of EtOH/water extract of AC, M + 2 %R— meatballs with 2% of extraction residue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

meatballs. The other ingredients (2%-AC and 1%-E) caused an even lower reduction of aw, 0.7 and 0.3%, respectively. Similarly, in the previous study (Tamkute et al., 2021) there was only a marginal effect on aw of pork products in the case of the cranberry pomace ethanol extract addition.

The state and content of myoglobin, storage temperature, pH, and packaging affect the colour of raw and cooked meatballs (Tamkute et al., 2021). The colour is also the first parameter that indicates the possible microbial or oxidative spoilage of meat products. The discolouration of red meat could occur due to the oxidation of the iron atoms in red oxy-haemoglobin (Peiretti et al., 2020). In the current study, all the rowanberry pomace-based ingredients decreased the lightness (L^*) of meatballs, likely due to the high content of anthocyanins in rowanberries (Sarv et al., 2021) (Fig. 3a). This kind of darkening of meat products has been previously mentioned for chokeberries, blueberries, grapes and blackcurrants (Peiretti et al., 2020; Tamkute et al., 2021). In the current case, the ingredients increased the redness (a^*) up to 48% (Fig. 3 b). The ingredients with higher amounts of bioactive components, such as 1%-E and 2%-AC, decreased the yellowness (b^*) of meatballs by 1.87% and 0.42%, respectively, but 2%-R increased b^* value by 0.51%, compared to the control, during 5 days of storage (Fig. 3 c).

3.4. Untargeted chemical profiling of meatballs added with rowanberry ingredients during storage

The untargeted UHPLC-Orbitrap analysis on the different meatballs allowed the putative annotation of 402 compounds according to their

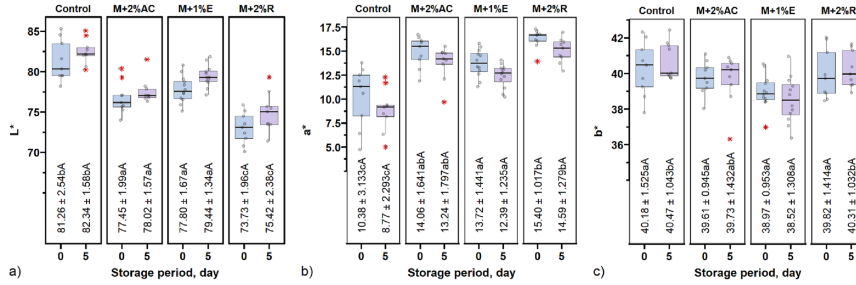


Fig. 3. Colour characteristics of meatballs (L*, a*, b*) on the day of preparation and after five days of storage Actual values are presented with grey dots; outliers are denoted with a red asterisk; mean values are presented as least-square means ± standard deviations; different lowercase letters indicate the statistical difference ($p < 0.05$) between the variants within the same day; different capital letters indicate the statistical difference ($p < 0.05$) between the days within the same variant. Control—without ingredients, M + 2 %AC— meatballs with 2% of defatted with supercritical CO_2 rowanberry pomace, M + 1 %E— meatballs with 1% of EtOH/water extract of AC, M + 2 %R— meatballs with 2% of extraction residue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

individual abundance values and composite mass spectra (MSMS). The number of chemical features annotated by untargeted metabolomics reflects the overall complexity of the meat matrix under investigation. A detailed list of all compounds annotated, with the corresponding mass spectra, isotopic profile, and identification-related information, is provided as **supplementary material**.

Afterwards, a multivariate statistical approach based on both unsupervised and supervised methods was used to group samples according to their similarity in the measured mass features. Firstly, the unsupervised hierarchical cluster analysis (HCA) was used to naïvely group samples according to intrinsic similarities in their chemical profile, and the corresponding heat-map (based on the Fold-Change, FC, and variations of each annotated compound) is reported in Fig. 4. The HCA consisted of two main groups: the first cluster hierarchically included the control samples at the different time-points of storage time (i.e., 0, 4, and 14 days), whilst the second cluster showed all the meatballs prepared with the different pomace ingredients (i.e., 2%-AC, 1%-E, and 2%-R). The heat-map highlighted the potential effect of rowanberry pomace on modifying the meat metabolomic profile. Similar separation trends were obtained by inspecting the unsupervised PCA score plot (**supplementary material**), highlighting a clear separation between the control samples and the meatballs with added ingredients along the first principal component (PC1). Looking at the unsupervised statistical findings, the impact of storage time was particularly evident when considering 2%-AC and 1%-E added samples. Therefore, to confirm the results highlighted by unsupervised multivariate methods, a supervised

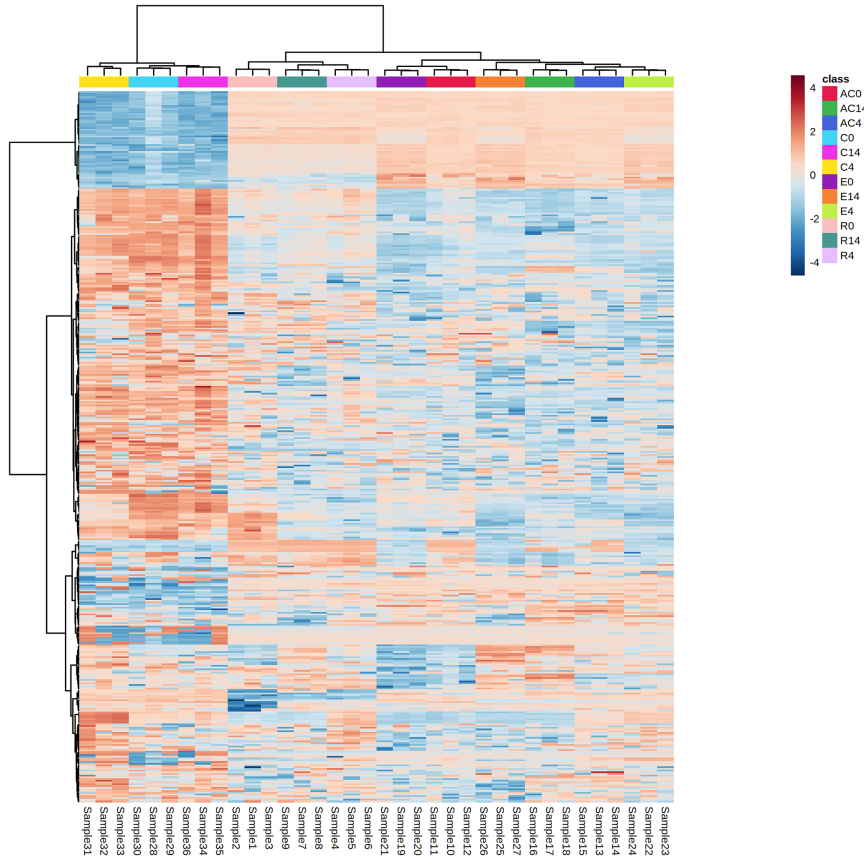


Fig. 4. Unsupervised hierarchical cluster analysis (HCA) considering the chemical profile of the different meatballs prepared with rowanberry functional ingredients (AC, E, and R) *vs* the control (C), at the different storage time-points (i.e., 0, 4, and 14 days).

orthogonal projection to latent structures discriminant analysis (OPLS-DA) was used to investigate the impact of storage time on the chemical profile of meatball samples. As shown from the OPLS-DA score plots reported in (**supplementary material**), each prediction model showed a clear separation trend between the different time points of storage time, recording more than acceptable goodness of fitting ($R^2Y > 0.9$) and prediction ability (Q^2 greater than 0.5) values. These prediction models confirmed the effectiveness of the OPLS-DA in predicting the major changes in the chemical composition of meatballs during storage at 4 °C. As the next step, we evaluated the changes in meat metabolites considering the last time point (i.e., 14 days), being more informative about the potential impact of oxidative processes on meat components and considering the protective role exerted by rowanberry pomace ingredients. Therefore, a new OPLS-DA model was built using only the meatball samples at 14 days of storage. As highlighted from the OPLS-DA score plot (Fig. 5), the control sample (C14) was separated from the added-samples (R14, E14, and AC14) along the orthogonal latent vector. Instead, meatballs with added rowanberry ingredients showed some differences in their chemical profile, with 2%-R and 1%-E samples found very close to each other. In contrast, a more characteristic chemical profile characterized the 2%-AC sample. Overall, the OPLS-DA model built consisted in excellent cross-validation and goodness parameters, with R^2X (cum) = 0.719, R^2Y (cum) = 0.993, and Q^2Y (prediction ability) = 0.919. Afterwards, the variable importance in projection (VIP) approach was used to select the most discriminant metabolites of the OPLS-DA model built. This approach revealed 184 discriminant metabolites having a VIP score higher than 1 (i.e., high prediction ability). These marker compounds are reported in **Table S1** (**supplementary material**), grouped in chemical classes provided by the comprehensive database FooDB. Additionally, we evaluated the Log Fold- Change (FC) variations between the three different treatments with the control. Looking at the discriminant markers reported in Table S1 (**supplementary material**), we found mainly terpenoids (52 compounds), amino acids (26 compounds), fatty acid derivatives (including esters, acids, and alcohols), polyphenols (16 compounds) and other compounds (e.g., aldehydes and ketones). Interestingly, fatty acid derivatives, aldehydes, and ketones were found to be up accumulated in the control sample compared to added-meatballs Table S1 (**supplementary material**). Overall, 1%-E was the most active rowanberry ingredient against the accumulation of aldehydes and ketones, recording cumulative LogFC values of 7.16 and 7.27, respectively, compared with the control (C) at 14 days of storage time. Regarding lipid oxidation phenomena, carbonyl compounds are described as a major by-product potentially affecting meat quality because of the off-flavours development due to the volatile fraction. Looking at our findings (**supplementary material**), the five discriminant aldehydic compounds were characterized by high LogFC values, such as 7-Dodecenal and 2,4-Heptadienal. This latter has already been detected as a marker associated with oxidative processes on meat components and its overall up-accumulation outlined a possible protective effect of the rowanberry pomace on lipid oxidation (Rocchetti, Lorenzo, et al., 2020). In this scenario, unsaturated lipids are chemically unstable and easily affected by degradation (Falowo et al., 2014). Our results revealed that linoleic acid derivatives showed an overall down-accumulation in the control sample (C), thus indicating a potentially higher lipidic peroxidation that was preserved by the addition of rowanberry pomace ingredients. However, looking at single marker compound, alpha-linolenic acid (i.e., one of the most involved in lipid peroxidation) was up-accumulated after 14 days only in meatballs added with 2%-R and 1%-E, while it showed a slight decrease when compared with the control (supplementary material). Another interesting result was related to the overall changes of glycerophospholipids in the three different pairwise comparisons under investigation Table S1 (**supplementary material**). In our experimental conditions, these compounds were characterized by an overall up-accumulation in the control sample, and the markers showing the higher values were phosphatidylethanolamine (PE) derivatives, such as PE (14:1(9Z)/14:1(9Z)), PE (14:0/14:0), PE (14:0/14:1(9Z)). Additionally, an up-accumulation of 25-Hydroxycholesterol was registered Table S1 (**supplementary material**); it is a steroid derivative showing high LogFC score values for each comparison under investigation (on average: 3.05). Its presence has been previously detected in two typical Italian pork products and is likely correlated to lipid and cholesterol oxidative processes (Novelli et al., 1997).

Regarding those discriminant terpenoids (including monoterpenoids, diterpenoids, triterpenoids, tetraterpenoids, and sesquiterpenoids) and phenolic compounds (mainly flavonoids and phenolic

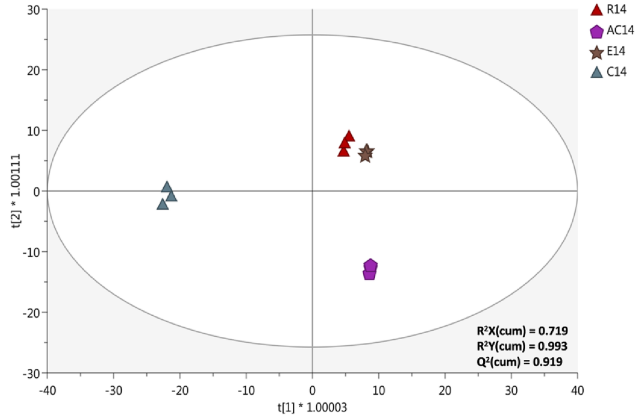


Fig. 5. Supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) considering the chemical profile of the different meatballs prepared with rowanberry functional ingredients (AC, E, and R) *vs* the control (C), at 14 days of the storage time period.

acids), most of the compounds were down accumulated in the control sample (C) and then clearly associated with the addition of rowanberry pomace ingredients in meatballs. Also, these secondary metabolites were characterized by similar LogFC values for each comparison against the control, thus showing a similar impact of the pomace ingredients during the storage time period (14 days). Several triterpenoid compounds of interest, such as 3-*trans-p*-coumaroylrotundic acid (previously detected as a biomarker of blueberry) (Das et al., 2022) and glycyrrhetinic acid (widely studied for its inflammatory properties) (Ming et al., 2013), have been identified following the addition of rowanberry ingredients. However, the main important phenolic compounds associated with adding rowanberry ingredients in meatballs were chlorogenic acid (average LogFC *vs* C = 13.98) and isoquercitrin (average LogFC *vs* C = 12.30).The distribution of these compounds in rowanberries is not novel, as well as their antioxidant activities against free radicals have been previously reported (Sarv et al., 2021). Therefore, the strong up-accumulation of these compounds at the end of storage time in meatballs added with pomace ingredients proved our hypothesis on greater protection against lipid oxidation phenomena.

4. Conclusions

In this work, natural ingredients possessing antioxidant capacity obtained by rowanberry pomace valorisation have been used as potential ingredients for meat products for preventing oxidation processes. By the day 5th, the meatballs with ingredients 2%-AC and 1%-E containing a higher amount of bioactives had decreased the yellowness (b^*) of meatballs. In the case of 1%-E, the pH of meatballs decreased, presumably due to the high concentration of chlorogenic acids, while in the case of 2%-R and 2%-AC, the pH remained stable, and the pH of the control sample increased. The increase in pH may indicate microbiological spoilage via the release of NH_3.

The PCA and OPLS-DA models were used to evaluate the chemical profiling of meatballs on days 0, 4 and 14. The results demonstrated the impact of storage time on changes in meatballs' chemical composition, especially in the case of 2%-AC and 1%-E added-samples with higher amounts of polyphenols.

In total, 184 discriminant metabolites were detected, consisting of 52 terpenoids, 26 amino acids, 16 polyphenols, aldehydes, and ketones, in addition to fatty acid derivatives. The most effective rowanberry ingredient against the development of unpleasant flavours caused by carbonyl compounds was 1%-E, at day-14 in the storage-time test. In addition, by the day-14 the concentration of linoleic acid derivatives had decreased only in the control sample (C).

Overall, these findings suggest the suitability of rowanberry pomace extract as a potential ingredient possessing antioxidant capacity for food products. In addition, the untargeted metabolomics can be used for assessing meat quality as well as evaluating the impact of antioxidants from rowanberry on the modifications of pork meatballs composition during their longer storage time. This approach allows finding possible correlations between natural by-products added to meat and its deterioration (mainly considering lipid peroxidation phenomena). Therefore, the approach gave the ideas for future studies, where the heating-induced effects on antioxidants must be considered. Moreover, for establishing the overall effects of various plant-based ingredients on the shelf-life of meat products, the microbiological characteristics should be determined in the future.

CRediT authorship contribution statement

Viive Sarv: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. **Kristi Kerner:** Formal analysis, Methodology. **Petras Rimantas Venskutonis:** Methodology, Supervision. **Gabriele Rocchetti:** Formal analysis, Methodology, Writing – original draft. **Pier Paolo Becchi:** Formal analysis, Methodology. **Luigi Lucini:** Methodology, Supervision. **Alo Tänavots:** Visualization. **Rajeev Bhat:** Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This work is supported by Plantvalor Project "PlantValor–full-scale product development service in synergy with the traditional activities of Polli Horticultural Research Centre 2020–2023", which has received funding from the European Regional Development Fund; and by ERA Chair in VALORTECH project, which has received funding from the European Union's Horizon 2020 Research and Innovation Program under grant agreement No. 810630. In addition, funding was received from Mobilitas Pluss ERA-Chair support (Grant No. MOBEC006 ERA Chair for Food (By-) Products Valorisation Technologies of the Estonian University of Life Sciences). The authors also acknowledge Estonian Ministry of Rural Affairs for the programmes "Collection, Conservation and Utilization of Plant Genetic Resources in 2021–2027". Gabriele Rocchetti acknowledges the project PON REACT EU DM 1062/21 (57-G-999-13) funded by the Ministero dell'Università e della Ricerca (MUR).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100761.

References

Aminzare, M., Hashemi, M., Ansarian, E., Bimkar, M., Azar, H. H., Mehrasbi, M. R., Daneshamooz, S., Raeisi, M., Jannat, B., & Afshari, A. (2019). Using natural antioxidants in meat and meat products as preservatives: A review. In *Advances in Animal and Veterinary Sciences (Vol. 7, Issue 5, pp. 417–426)*. Nexus Academic Publishers. https://doi.org/10.17582/journal.aavs/2019/7.5.417.426.

Aziz, M., & Karboune, S. (2018). Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition, 58*(3), 486–511. https://doi.org/10.1080/10408398.2016.1194256

Babaoğlu, A. S., Unal, K., Dilek, N. M., Poçan, H. B., & Karakaya, M. (2022). Antioxidant and antimicrobial effects of blackberry, black chokeberry, blueberry, and red currant pomace extracts on beef patties subject to refrigerated storage. *Meat Science, 187*. https://doi.org/10.1016/j.meatsci.2022.108765

Barcenilla, C., Ducic, M., López, M., Prieto, M., & Álvarez-Ordóñez, A. (2022). Application of lactic acid bacteria for the biopreservation of meat products: A systematic review. *Meat Science, 183*. https://doi.org/10.1016/J.MEATSCI.2021.108661

Beale, D. J., Kouremenos, K. A., & Palombo, E. A. (2016). Microbial metabolomics: Applications in clinical, environmental, and industrial microbiology. In *Microbial Metabolomics: Applications in Clinical, Environmental, and Industrial Microbiology*. Springer International Publishing. 10.1007/978-3-319-46326-1.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*, 25–30.

Das, P. R., Darwish, A. G., Ismail, A., Haikal, A. M., Gajjar, P., Balasubramani, S. P., … El-Sharkawy, I. (2022). Diversity in blueberry genotypes and developmental stages enables discrepancy in the bioactive compounds, metabolites, and cytotoxicity. *Food Chemistry, 374*. https://doi.org/10.1016/j.foodchem.2021.131632

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. In *Antioxidants* (Vol. 8, Issue 10). MDPI. 10.3390/antiox8100429.

Dussault, D., Vu, K. D., & Lacroix, M. (2014). In vitro evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. *Meat Science, 96*(1), 514–520. https://doi.org/10.1016/j.meatsci.2013.08.015

Falowo, A. B., Fayemi, P. O., & Muchenje, V. (2014). Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. In *Food Research International* (Vol. 64, pp. 171–181). Elsevier Ltd.. https://doi.org/10.1016/j.foodres.2014.06.022

Fiehn, O. (2002). Metabolomics-the link between genotypes and phenotypes. In *Plant Molecular Biology* (Vol. 48).

Folin, O., & Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *The Journal of Biological Chemistry, 2*, 627–650.

Gallego, M. G., Gordon, M. H., Segovia, F. J., & Almajano, M. P. (2015). Caesalpinia decapetala Extracts as Inhibitors of Lipid Oxidation in Beef Patties. *Molecules, 20*(8), 13913–13926. https://doi.org/10.3390/molecules200813913

Ganhão, R., Estévez, M., Kylli, P., Heinonen, M., & Morcuende, D. (2010). Characterization of selected wild mediterranean fruits and comparative efficacy as inhibitors of oxidative reactions in emulsified raw pork burger patties. *Journal of Agricultural and Food Chemistry, 58*(15), 8854–8861. https://doi.org/10.1021/jf101646y

Halagarda, M., & Wójciak, K. M. (2022). Health and safety aspects of traditional European meat products. A review. *Meat Science, 184*. https://doi.org/10.1016/j.meatsci.2021.108623

Hallmann, E., Orpel, E., & Rembiałkowska, E. (2011). The content of biologically active compounds in some fruits from natural state. *Vegetable Crops Research Bulletin, 75*(1), 81–90. https://doi.org/10.2478/v10032-011-0020-8

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*. https://doi.org/10.1021/jf990146l

Kerner, K., Jõudu, I., Tänavots, A., & Venskutonis, P. R. (2021). Application of raw and defatted by supercritical co2 hemp seed press-cake and sweet grass antioxidant extract in pork burger patties. *Foods, 10*(8). https://doi.org/10.3390/foods10081904

Lorenzo, J. M., Pateiro, M., Domínguez, R., Barba, F. J., Putnik, P., Kovačević, D. B., … Franco, D. (2018). Berries extracts as natural antioxidants in meat products: A review. *Food Research International, 106*, 1095–1104. https://doi.org/10.1016/j.foodres.2017.12.005

Mena, B., Fang, Z., Ashman, H., Hutchings, S., Ha, M., Shand, P. J., & Warner, R. D. (2020). Influence of cooking method, fat content and food additives on physicochemical and nutritional properties of beef meatballs fortified with sugarcane fibre. *International Journal of Food Science and Technology, 55*(6), 2381–2390. https://doi.org/10.1111/ijfs.14482

Minato Nakazawa, M. (2022). *Package "fmsb" Title Functions for Medical Statistics Book with some Demographic Data Depends R (>= 2.2.0)* (pp. 1–66). https://minato.sip21c.org/msb/.

Ming, J., Yoke, C., & Yin, A. (2013). Therapeutic Effects of Glycyrrhizic Acid. *Natural Product Communications, 8*(3), 415–418.

Mokrzycki, W. S., & Tatol, M. (2012). Color difference Delta E-A survey Colour difference ΔE-A survey. *Machine Graphics and Vision*, 1–28. https://www.researchgate.net/publication/236023905.

Munekata, P. E. S., Rocchetti, G., Pateiro, M., Lucini, L., Domínguez, R., & Lorenzo, J. M. (2020). Addition of plant extracts to meat and meat products to extend shelf-life and health-promoting attributes: An overview. In *Current Opinion in Food Science* (Vol. 31, pp. 81–87). Elsevier Ltd.. https://doi.org/10.1016/j.cofs.2020.03.003

Novelli, E., Zanardi, E., Ghiretti, G. P., Campanini, G., Dazzi, G., Madarena, G., & Chizzolini, R. (1997). Lipid and Cholesterol Oxidation in Frozen Stored Pork, Salame Milano and Mortadella. *Meat Science, 48*(112), 29–40.

Paglarini, C. de S., Vidal, V. A. S., Martini, S., Cunha, R. L., & Pollonio, M. A. R. (2022). Protein-based hydrogelled emulsions and their application as fat replacers in meat products: A review. In *Critical Reviews in Food Science and Nutrition* (Vol. 62, Issue 3, pp. 640–655). Taylor and Francis Ltd. 10.1080/10408398.2020.1825322.

Pateiro, M., Vargas, F. C., Chincha, A. A. I. A., Santana, A. S., Strozzi, I., Rocchetti, G., Barba, F. J., Domínguez, R., Lucini, L., do Amaral Sobral, P. J., & Lorenzo, J. M. (2018). Guarana seed extracts as a useful strategy to extend the shelf life of pork patties: UHPLC-ESI/QTOF phenolic profile and impact on microbial inactivation, lipid and protein oxidation and antioxidant capacity. *Food Research International, 114*, 55–63. https://doi.org/10.1016/j.foodres.2018.07.047

Peiretti, P. G., Gai, F., Zorzi, M., Aigotti, R., & Medana, C. (2020). The effect of blueberry pomace on the oxidative stability and cooking properties of pork patties during chilled storage. *Journal of Food Processing and Preservation, 44*(7). https://doi.org/10.1111/jfpp.14520

Rocchetti, G., Bernardo, L., Pateiro, M., Barba, F. J., Munekata, P. E. S., Trevisan, M., … Lucini, L. (2020). Impact of a pitanga leaf extract to prevent lipid oxidation processes during shelf life of packaged pork burgers: An untargeted metabolomic approach. *Foods, 9*(11). https://doi.org/10.3390/foods9111668

Rocchetti, G., Lorenzo, J., Barba, F., Munekata, P., Bernardo, L., Tomasevic, I., … Lucini, L. (2020). Untargeted metabolomics to explore the oxidation processes during shelf life of pork patties treated with guarana seed extracts. *International Journal of Food Science and Technology, 55*(3), 1002–1009. https://doi.org/10.1111/ijfs.14329

Rocchetti, G., Michelini, S., Pizzamiglio, V., Masoero, F., & Lucini, L. (2021). A combined metabolomics and peptidomics approach to discriminate anomalous rind inclusion levels in Parmigiano Reggiano PDO grated hard cheese from different ripening stages. *Food Research International, 149*. https://doi.org/10.1016/j.foodres.2021.110654

Salek, R. M., Steinbeck, C., Viant, M. R., Goodacre, R., & Dunn, W. B. (2013). The role of reporting standards for metabolite annotation and identification in metabolomic studies. *GigaScience, 2*(1). https://doi.org/10.1186/2047-217X-2-13

Sarv, V., Venskutonis, P. R., Rätsep, R., Aluvee, A., Kazernavičiūtė, R., & Bhat, R. (2021). Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (Sorbus aucuparia L.) Cultivated in Estonia. *Antioxidants, 10*(11), 1779. https://doi.org/10.3390/antiox10111779

Searle, S. R., Speed, F. M., & Milliken, G. A. (1980). Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. *The American Statistician, 34*(4), 216–221. https://doi.org/10.1080/00031305.1980.10483031

Tamkute, L., Vaicekauskaitė, R., Melero, B., Jaime, I., Rovira, J., & Venskutonis, P. R. (2021). Effects of chokeberry extract isolated with pressurized ethanol from defatted pomace on oxidative stability, quality and sensory characteristics of pork meat products. *LWT, 150*. https://doi.org/10.1016/j.lwt.2021.111943

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., … Arita, M. (2015). MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nature Methods, 12*(6), 523–526. https://doi.org/10.1038/nmeth.3393

Tsugawa, H., Kind, T., Nakabayashi, R., Yukihira, D., Tanaka, W., Cajka, T., … Arita, M. (2016). Hydrogen Rearrangement Rules: Computational MS/MS Fragmentation and Structure Elucidation Using MS-FINDER Software. *Analytical Chemistry, 88*(16), 7946–7958. https://doi.org/10.1021/acs.analchem.6b00770

Venskutonis, P. R. (2020). Berries. In *Valorization of Fruit Processing By-products* (pp. 95–125). Elsevier. https://doi.org/10.1016/b978-0-12-817106-6.00005-8.

Wichchukit, S., & O'Mahony, M. (2015). The 9-point hedonic scale and hedonic ranking in food science: Some reappraisals and alternatives. *Journal of the Science of Food and Agriculture, 95*(11), 2167–2178. https://doi.org/10.1002/jsfa.6993

CURRICULUM VITAE

First name Viive
Surname Sarv
Date of birth 27.05.1960

E-mail viive.sarv@emu.ee
ORCID 0000-0002-6241-8475

Education

2019–2023	PhD student in Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Horticulture
2014–2017	MASc Degree, University of Toronto, 2017
1978–1983	BSc in Chemistry, University of Tartu, 1983, now equal to European MSc degree

Career history

2022–...	Estonian University of Life Sciences, Institute of Institute of Agricultural and Environmental Sciences, Chair of Horticulture, junior researcher in Polli Horticultural Research Centre
2018–...	Estonian University of Life Sciences, Institute of Institute of Agricultural and Environmental Sciences, Chair of Horticulture, specialist in Polli Horticultural Research Centre
2014–2017	University of Toronto, research assistant in Food engineering laboratory

Field of research

1. Biosciences and Environment; 1.1. Biochemistry; 1.7. Food Sciences
CERCS B191 Plant biochemistry; T430 Food and drink technology

Participation in research projects

2023 ”Development of blackcurrant marmalades”, Viive Sarv, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Horticulture, Polli Horticultural Research Centre

2022–2024 “Pre-breeding strategies for obtaining new resilient and added value berries”, Ave Kikas, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Horticulture, Polli Horticultural Research Centre

2020−2023 “PlantValor – full-scale product development service in synergy with the traditional activities of Polli Horticultural Research Centre”, Hedi Kaldmäe, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre

2019−2023 “Feeze drying. Development of the pre-treatment technologies of freeze-drying and valorisation of the press residues”, Peeter Laurson, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre

2018−2023 “ERA-Chair for Food (By-) Products Valorisation Technologies of the Estonian University of Life Sciences”, Ivi Jõudu; Piia Pääso; Rajeev Bhat, Estonian University of Life Sciences

2020−2023 “Technological possibilities for the production of concentrates and clarified juices based on local raw materials”, Uko Bleive, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Polli Horticultural Research Centre

2021−2023 “Development of raspberry seed valorisation technology and product prototype”, Ave Kikas, Estonian University of Life Sciences, Institute of Agricultural and

Environmental Sciences, Polli Horticultural Research Centre

2021–2023 "Utilization of bioactive components of production residues of plant foods for increasing of durability and healthiness of animal food and for valorization of animal origin food" (RESTA14), Reelika Rätsep, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences

2019–2022 "Separation, concentration and characterisation of properties of the plant proteins", Peeter Laurson, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences

2021–2023 "Valorization of beer production residue", Uko Bleive, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences

2020–2021 "Development of plant-based ice-ceam powder and evaluation of composed ice-creams", Viive Sarv, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences

2020 "Blackcurrant seed oil production solution from juice pressing residues", Uko Bleive, Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences

Honours & awards

2023 Garage 48 Food, the award in hackaton for development reduced fat meatballs, project "Moat", project team: Monica Nabil Gayed Ibrahim, Kristi Kerner, Viive Sarv, Karl-Gustav Gimbutas

Teaching Antioxidants, determination of antioxidant activity

Self-improvement courses

"Biomass to Sustainable Bio-products ", organized by the ERA Chair in VALORTECH in the Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, March 29-31.2023

The workshop/practical training on edible films and coating as sustainable food packaging solutions, organized by the ERA Chair in VALORTECH in the Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Tartu, Nov. 03.2022.

Erasmus K2 Project in University of Calabria, Rende, Italy: "Strategies regarding the valorization of horticultural and agricultural by-products as functional foods in the context of a circular economy" 25.09-01.10.2022

"Agri-food wastes and by-products valorization: Functional product development" organized by the ERA Chair in VALORTECH in the Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences 19.09 - 21.09.2022

"Application of Immobilized Enzymes in Food Industry and Food Waste Valorization", organized by the ERA Chair in VALORTECH in the Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, 25.01.2022

"Food Waste Valorization: Natural Pigments Perspectives" organized by ERA-Chair for Food By-products Valorization Technologies, Estonian University of Life Sciences, 28.10.2021

"Valorization of Food Industry Wastes and By-products", organized by the ERA Chair in VALORTECH in the Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences 28.-30.09.2021

"Valorisation of Vegetal Waste and By-products: An eminent source of Natural Bioactive Ingredients", organized by the ERA Chair in VALORTECH in the Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, 1 ECTS, 11.12.2020

"Workshop on Principles and Techniques for the Extraction of Bioactive Compounds", organized by the ERA Chair in VALORTECH in the Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, 2 ECTS, 08.-09.10.2020

ELULOOKIRJELDUS

Eesnimi Viive
Perenimi Sarv
Sünniaeg 27.05.1960

E-mail viive.sarv@emu.ee
ORCID 0000-0002-6241-8475

Haridustee

2019–2023 Eesti Maaülikool, Põllumajandus ja keskkonna instituut, Aianduse õppetool, doktoriõpe

2014–2017 Toronto Ülikool, Insenerikeemia teaduskond, magistrikraad rakendusteaduste erialal

1978–1983 Tartu Ülikool, Füüsika-keemia teaduskond, bakalaureuse kraad keemia erialal, nüüdsest vastav Euroopa magistrikraadile

Töökohad

2022–... Eesti Maaülikooli Polli Aiandusuuringute Keskuse nooremteadur

2018–2022 Eesti Maaülikooli Polli Aiandusuuringute Keskuse spetsialist

2014–2017 Toronto Ülikooli, Insenerikeemia ja rakendusteduste teaduskonna, Toidutehnoloogia laboratootiumi õppetöö assistant

Teadustöö põhisuunad

1. Bio- ja keskkonnateadused; 1.7. Toiduteadused
1. Bio- ja keskkonnateadused; 1.1. Biokeemia;
CERCS B191 Taimebiokeemia; T430 Toiduainete ja jookide tehnoloogia

Osalemine teadus- ja arendusprojektides

2023 „Mustsõstra marmelaadide arendus“, Viive Sarv, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2022–2024 “Eelaretuse strateegiad uute plastiliste ja lisandväärtusega marjasortide saamiseks”, Ave Kikas, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2020−2023 “PlantValor – terviklik tootearendusteenus sünergias Polli aiandusuuringute keskuse traditsiooniliste tegevusvaldkondadega”, Hedi Kaldmäe, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2019−2023 “KÜLMKUIVATUS. Külmkuivatamise eeltöötlemis-tehnoloogiate arendamine ja pressimisjääkide väärindamine”, Peeter Laurson, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2018−2023 Eesti Maaülikooli “ERA-õppetool ValorTech”, Ivi Jõudu; Piia Pääso; Rajeev Bhat

2020−2023 “Kohalikul toorainel põhinevate kontsentraatide ja klaaritatud mahlade tootmise tehnoloogilised võimalused”, Uko Bleive, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2021−2023 “Vaarikaseemnete väärindamise tehnoloogia ja toote prototüübi arendus”, Ave Kikas, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2021−2023 “Taimsete tootmisjääkide bioaktiivsete komponentide kasutamine loomsete toiduainete säilivuse ja tervislikkuse suurendamiseks või loomsete toitude väärindamiseks (TAIMLOOMTOIT)” (RESTA14), Reelika Rätsep,

Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2019−2022 "Taimsete valkude eraldamine, kontsentreerimine ja omaduste iseloomustamine", Peeter Laurson, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2021−2023 "Õlleraba väärindamine", Uko Bleive, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2020−2021 "Taimsete jäätisepulbrite arendus ja koostatud jäätiste hindamine", Viive Sarv, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

2020 "Musta sõstra seemneõli tootmislahendus mahla pressimisjääkidest", Uko Bleive, Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

Õppetöö Antioksüdantsuse määramise võimalused ja näiteid antioksüdantide kasutamisvõimalustest **(**VL.1331 Toiduainete töötlemisel tekkivate kõrvalsaaduste väärindamise ainekava raames)

Enesetäiendused

Koolitus "Biomass to Sustainable Bio-products ", korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 29-31.03.2023.

Töötuba/ koolitus söödavate kilede ja katete kasutamine jätkusuutlike toidupakenditena, korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 3.11.2022.

Erasmus K2 koolitus/ õppereis teemal "Strategies regarding the valorization of horticultural and agricultural by-products as functional

foods in the context of a circular economy" Calabria Ülikool, Rende, Itaalia, 25.09–01.10.2022.

Koolitus "Agri-food wastes and by-products valorization: Functional product development", korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 19.–21.09.2022

Koolitus "Application of Immobilized Enzymes in Food Industry and Food Waste Valorization", korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 25.01.2022.

Koolitus "Food Waste Valorization: Natural Pigments Perspectives" korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 28.10.2021.

Koolitus "Valorization of Food Industry Wastes and By-products", korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 28.–30.09.2021.

Koolitus "Valorisation of Vegetal Waste and By-products: An eminent source of Natural Bioactive Ingredients", korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 1 ECTS, 11.12.2020.

Töötuba "Principles and Techniques for the Extraction of Bioactive Compounds", korraldaja ERA õppetool VALORTECH, Veterinaarmeditsiini ja loomakasvatuse instituut, Eesti Maaülikool, Tartu, 2 ECTS, 08.–09.10.2020.

Autasud

2023 Garage 48 Food, auhind toiduteemalisel häkatonil vähendatud rasvasisaldusega lihapallide arenduse eest, projekt "Moat", projekti liikmed: Monica Nabil Gayed Ibrahim, Kristi Kerner, Viive Sarv, Karl-Gustav Gimbutas

LIST OF PUBLICATIONS

1.1. Scholarly papers indexed by Web of Science Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index and/or indexed by Scopus

Sarv, Viive; Kerner, Kristi; Venskutonis, Petras, Rimantas; Rocchetti, Gabriele; Becchi, Pier Paolo; Lucini, Luigi; Tänavots, Alo; Bhat, Rajeev, 2023. Untargeted metabolomics and conventional quality characterization of rowanberry pomace ingredients in meatballs. Food Chemistry: X, 19, 100761. doi.org/10.1016/j.fochx.2023.100761

Rocchetti, Gabriele; Ferronato, Giulia; **Sarv, Viive**; Kerner, Kristi; Venskutonis, Petras Rimantas; Lucini, Luigi, 2023. Meat extenders from different sources as protein-rich alternatives to improve the technological properties and functional quality of meat products. Current Opinion in Food Science, 49, 100967. DOI: 10.1016/j.cofs.2022.100967.

Sarv, Viive; Venskutonis, Petras Rimantas; Rätsep, Reelika; Aluvee, Alar; Kazernaviciute, Rita; Bhat, Rajeev., 2021. Antioxidants Characterization of the Fruit, Juice, and Pomace of Sweet Rowanberry (*Sorbus aucuparia* L.) Cultivated in Estonia. Antioxidants, 10 (11), ARTN 1779. DOI: 10.3390/antiox10111779.

Sarv, Viive; Venskutonis, Petras Rimantas; Bhat, Rajeev, 2020. The *Sorbus* spp. Underutilised Plants for Foods and Nutraceuticals: Review on Polyphenolic Phytochemicals and Antioxidant Potential, Antioxidants, 9 (9), ARTN 8131. DOI: 10.3390/antiox9090813.

Sarv, Viive; Trass, Olev; Diosady, Levente L., 2017. Preparation and Characterization of Camelina sativa Protein Isolates and Mucilage, Journal of the American Oil Chemists' Society, 94 (10), 1279−1285. DOI: 10.1007/s11746-017-3031-x.

2.5. Published reports of a scientific project or a scientific analyse

Laurson, Peeter; Kaldmäe, Hedi; **Sarv, Viive**, 2022. Taimsete valkude eraldamine, kontsentreerimine ja omaduste iseloomustamine. Eesti

Maaülikool, Põllumajandus- ja keskkonnainstituut, Aianduse õppetool, Polli Aiandusuuringute Keskus

3.5. Papers/presentations, published in local conference proceedings

Rätsep, Reelika; Kaldmäe, Hedi; Bleive, Uko; **Sarv, Viive**; Anton, Dea; Püssa, Tõnu; Roasto, Mati; Venskutonis, Petras Rimantas, 2022. Mahlapressimisjääkidest valmistatud pulbrite bioaktiivne potentsiaal, Conference proceedings: Healthy Animal and Healthy Food, Estonian University of Life Sciences.

Kerner, Kristi; **Sarv**, **Viive**; **Tänavots,** Alo; Venskutonis, Petras Rimantas, 2023. Pihlakamarjade pressjäägi kasutamine sealihast lihapallides, Conference proceedings: Healthy Animal and Healthy Food, Estonian University of Life Sciences.

5.2. Conference theses

Sarv, Viive; Kerner, Kristi; Venskutonis, Petras Rimantas; Rätsep, Reelika; Rocchetti, Gabriele; Becchi, Pier Paolo; Lucini, Luigi; Tänavots, Alo; Bhat, Rajeev, 2022. Rowan fruit pomace as functional ingredient in meatballs formulation. Proceedings of 14th International Conference and Exhibition on Nutraceuticals and Functional Foods, October 2-5, 2022, İstanbul, Turkey

Sarv, **Viive;** Venskutonis, Petras Rimantas; Tamkutė, Laura; Baranauskienė, Renata; Urbonavičienė, Dalia; Viškelis, Pranas and Bhat, Rajeev, 2022**.** Fractionation of lipophilic components from rowanberry pomace by SFE-CO2 extraction and separation at suncritical conditions, Proceedings of Green Extraction of Natural Products GENP 2022 POREČ, CROATIA 27 and 28 October 2022 Valamar Diamant Hotel Poreč, Croatia

Sarv, Viive; Kerner, Kristi; Venskutonis, Petras Rimantas; Rocchetti, Gabriele; Becchi, Pier Paolo, Lucini, Luigi; Tänavots, Alo; Bhat, Rajeev, 2023, Evaluation of rowan fruit pomace ingredients in meatballs by conventional quality characterization and UHPLC-QTOF-MS based untargeted metabolomics with multivariate data analysis, Abstract Book of FOODBALT 2023 16th Baltic Conference on Food Science and

Technology " Traditional meets non-traditional in future food", May 11–12, 2023, Jelgava, Latvia

Sarv, **Viive;** Venskutonis, Petras Rimantas; Tamkutė, Laura; Baranauskienė Renata; Urbonavičienė, Dalia; Viškelis, Pranas and Bhat, Rajeev, 2023**.** Extraction of lipophilic components from rowanberry pomace with supercritical CO_2 and their fractionation at subcritical conditions, Proceedings of the 19th European Meeting of Supercritical Fluids May 21–24, 2023 Budapest, Hungary

Kristi Kerner, **Viive Sarv**, Ivi Jõudu, Alo Tänavots, Petras Rimantas Venskutonis, 2023, Effect of blackcurrant skin ingredients on the physicochemical properties of pork meatballs, Proceedings of **EuroFoodChem XXII June 14-16, 2023 Belgrade, Serbia**

6.4. Popular sciences books

Arus, Liina; Rätsep, Reelika; Vahenurm, Mailis; **Sarv, Viive**; Zimmer, Elmar, 2022. Väike toompihlakaraamat, Vali Press OÜ.

Arus, Liina; Rätsep, Reelika; **Sarv, Viive**; Zimmer, Elmar, 2022. Väike arooniaraamat, Vali Press OÜ.

Arus, Liina; Rätsep, Reelika; **Sarv, Viive**; Zimmer, Elmar, 2022. Väike leedriraamat, Vali Press OÜ.

Arus, Liina; Rätsep, Reelika; **Sarv, Viive**; Zimmer, Elmar, 2023. Väike lodjapuuraamat, Vali Press OÜ.

VIIS VIIMAST KAITSMIST

KEYVAN ESMAEILZADEH SALESTANI
MULLA MIKROBIOOM JA ODRA TRANSKRIPTOOM ERI VILJELUSVIISIDES
IMPACT OF CROPPING SYSTEMS ON SOIL MICROBIOME AND BARLEY TRANSCRIPTOME
Dotsent Evelin Loit
16. juuni 2023

MERLE VALDMANN
TSÜTOLOOGILINE ENDOMETRIIT PIIMALEHMADEL: DIAGNOOSIMINE, MÕJU SIGIVUSELE JA PRAAKIMISELE NING SEOSED METABOOLSE STRESSIGA
CYTOLOGICAL ENDOMETRITIS IN DAIRY COWS: DIAGNOSTICS, EFFECTS ON FERTILITY AND CULLING, AND ASSOCIATIONS WITH METABOLIC STRESS
Professor Andres Valdmann
22. august 2023

HANNES TAMME
PUIDU KUIVATUSE KONTROLLI JA OPTIMEERIMISE MEETODITE ARENDAMINE
DEVELOPMENT OF CONTROL AND OPTIMIZATION METHODS FOR WOOD DRYING
Professor Emeritus Peeter Muiste, PhD, teadur Valdek Tamme
30. august 2023

RAUL SAMPAIO DE LIMA
MEHITAMATA ÕHUSÕIDUKITE UUDSED RAKENDUSED TULEOHJESÜSTEEMIDE TÄIUSTAMISEL
NOVEL APPROACHES IN MULTI-SENSOR UNMANNED AERIAL VEHICLES AS BASIS FOR ENHANCING FIRE MANAGEMENT FRAMEWORKS
Professor Kalev Sepp, professor Mait Lang, PhD Ants Vain
2. november 2023

HASSAN YUSUF SULAIMAN
QUANTITATIVE IMPACTS OF INTERACTIVE BIOTIC AND ABIOTIC STRESSES ON PLANT PERFORMANCE: STRESS RESPONSES, PRIMING, AND ACCLIMATION. KOOSESINEVATE BIOOTILISTE JA ABIOOTILISTE STRESSIDE MÕJU TAIMEDELE: STRESSIVASTUSTEST KOHANEMISENI.
Professor Ülo Niinemets
16.november 2023

ISSN 2382-7076
ISBN 978-9916-719-36-7 (trükis)
ISBN 978-9916-719-37-4 (pdf)