DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

Phenolic Compounds from Finnish Berry Species to Enhance Food Safety

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In memory of my grandparents

TABLE OF CONTENTS

ABSTRACT ..... **i**

TIIVISTELMÄ ..... **iii**

LIST OF ABBREVIATIONS ..... **v**

LIST OF ORIGINAL PUBLICATIONS ..... **vii**

1 INTRODUCTION **1**

2 REVIEW OF THE LITERATURE **3**

2.1 Phenolic compounds in berry species 3
2.1.1 Phenolic profiles of berries 3
2.1.2 Phenolic profiles of leaves 6
2.2 Anti-oxidative activity of extracts of berry species 8
2.3 Structure-antioxidant activity relationship of phenolic compounds 19
2.3.1 Phenolic acids 19
2.3.2 Flavonoids 21
2.3.3 Tannins 24
2.4 Anti-bacterial activities of extracts of berry species 33
2.5 Mechanism of anti-bacterial activities of phenolics and structure-activity relationship 48
2.6 Summary and future prospects 50

3 AIMS OF THE STUDY **52**

4 MATERIALS AND METHODS **53**

4.1 Plant materials 54
4.2 Sample preparation 56
4.2.1 Extraction of phenolic compounds (Study I, III, and IV) 56
4.2.2 Isolation of unknown compounds (Study I) 56
4.2.3 Fractionation of phenolic compounds (Study III) 56
4.3 Identification and quantification of phenolic compounds 57
4.3.1 Liquid chromatography analysis (Study I, III, and IV) 57
4.3.2 Mass spectrometric analysis (Study I, III, and IV) 57
4.3.3 Nuclear magnetic resonance analysis (Study I and III) 58
4.4 *In vitro* assays of anti-oxidative activities (Study II and III) 58
4.5 *In vitro* study of anti-bacterial activities (Study II and III) 59
4.6 Statistical analyses 59

5 RESULTS AND DISCUSSION 60

5.1 Phenolic profiles in Finnish berry species 60
5.1.1 Phenolic composition in extracts of Finnish berry species (Study I) 60
5.1.2 Phenolic composition in fractions of Finnish berry species (study III) 62
5.1.3 Phenolic composition in berries of blackcurrant cultivars (Study IV) 65
5.2 Anti-oxidative activities of Finnish berry species 67
5.2.1 Anti-oxidative activity of phenolic extracts (Study II) 67
5.2.2 Anti-oxidative activity of fractions of phenolic extracts (Study III) 70
5.2.3 Correlation of phenolic compounds with anti-oxidative activity (Study II and III) 71
5.2.3.1 Multivariate correlation between phenolics and anti-oxidative activities 71
5.2.3.2 Bivariate correlation between phenolics and anti-oxidative activities 76
5.3 Anti-bacterial activities of Finnish berry species 80
5.3.1 Anti-bacterial activity of phenolic extracts (Study II) 80
5.3.2 Anti-bacterial activity of fractions of phenolic extracts (Study III) 82
5.3.3 Correlation of phenolic compounds with anti-bacterial activity (Study II and III) 85
5.4 Variation of phenolic profiles among cultivars and growing years (study IV) 88

6 SUMMARY AND CONCLUSION 95

ACKNOWLEDGEMENTS 96

REFERENCES 98

APPENDIX: ORIGINAL PUBLICATIONS 109

ABSTRACT

Food safety is of worldwide importance and in close relation to human daily life and wellbeing. Preservatives are often necessary to ensure the shelf life of food products. Despite the generally proven safety of synthetic food additives, there is an increasing demand for natural food preservatives due to the preference for natural foods by the consumers.

Fruits and leaves of berry plants contain a large group of phenolic compounds as secondary metabolites. These compounds have anti-microbial and anti-oxidative functions. There is potential to produce natural food-preservatives using berry and leaf extracts of different berry species.

The aims of this research were: 1) to determine phenolic profiles of food grade water-ethanol extracts of leaves and fruits of thirteen Finnish berry-bearing plants; 2) to evaluate their anti-oxidative activities and antimicrobial effects on foodborne pathogens; 3) to study the influence of genotype (cultivars) and annual variation on phenolic profiles of berries among 21 cultivars of *Ribes nigrum.*

The total content of phenolic compounds was significantly higher in aqueous-ethanol extracts of the leaves than in the corresponding extracts of the berries (8–71 vs. 54–786 mg/100 mL). Sea buckthorn leaves had the highest total content of phenolics (606–786 mg/100 mL) due to the abundance of ellagitannins. In the leaf extract of lingonberry, *β-p*-Arbutin accounted for over 40 % of the total phenolics (271 mg/100 mL), followed by (+)-catechin, procyanidins, and quercetin glycosides. The leaf extract of bilberry was rich in caffeoylquinic acid (80 % of the total content of phenolics). Anthocyanins formed the most dominant group of phenolic compounds in the dark-skinned berries, whereas sea buckthorn berries contained mostly isorhamnetin glycosides.

There was considerable variation in both anti-oxidative and anti-bacterial activities among the extracts with strong correlations with the total content of phenolics. Flavonoids correlated strongly with the activities measured with Folin-Ciocalteu, oxygen radical absorbance capacity (ORAC), and total radical trapping antioxidant parameter (TRAP) assays. The correlation was especially strong between the anti-oxidative activity and the content of proanthocyanins (procyanidin dimers and trimers), flavan-3-ols ((+)-catechin and (-)-epicatechin), and glycosylated flavonols (quercetins). Anthocyanins and non-flavonoid phenolic compounds correlated highly to the activity of scavenging DPPH radicals. Non-flavonoid phenolic compounds had major contribution to inhibition of the growth of some bacterial species, example of which is the

correlation between content of ellagitannins and inhibitory capacity against *Staphylococcus aureus* and *Bacillus cereus* strains.

Eight extracts of fruits and leaves were chosen for fractionation using Sephadex LH-20 column, and the anti-oxidative and anti-microbial activities of the fractions were further studied in order to pinpoint the major phenolics contributing to these activities. The results suggested that ORAC activities of quercetin glycosides might decrease with increasing number of sugar moieties. For mono-glycosylquercetins, the nature of sugar moieties might also influence the capacity of quenching peroxyl-radicals. Compared to *S. aureus* strains, *Escherichia coli* showed a higher resistance to phenolics in the fractions studied.

The content and constituent of phenolics in blackcurrant berries differed significantly across cultivars and the studied growing years. The varying concentration of phenolic acid derivatives was the major compositional diversities among the cultivars (cultivated in the same location) originating from Scotland, Lithuania, and Finland. The cultivars of the same origin were grouped based on the concentration of 3-*O*-glycosides of delphinidin and cyanidin. The berries harvested in the two studied years differed in the concentration of phenolic acid conjugates and glycosylated quercetins.

TIIVISTELMÄ

Elintarviketurvallisuus on maailmanlaajuisesti tärkeää ja kytköksissä ihmisten päivittäiseen elämään ja hyvinvointiin. Säilöntäaineiden käyttö on usein välttämätöntä tuotteilta vaadittavan hyllyiän takaamiseksi. Huolimatta siitä, että elintarvikkeiden synteettiset lisäaineet on osoitettu yleisesti turvallisiksi, on tunnistettu kuluttajien mieltymyksistä johtuva tarve kehittää luontaisia säilöntäaineita.

Marjakasvien hedelmät ja lehdet sisältävät laajan kirjon fenolisia yhdisteitä, jotka ovat kasvin sekundaarisia aineenvaihduntatuotteita. Näillä yhdisteillä on antimikrobisia ja hapettumiselta suojaavia vaikutuksia. On mahdollista valmistaa luontaisia elintarvikesäilöntäaineita monien marjalajien lehti- ja marjauutteista.

Tämän tutkimuksen tavoitteina oli: 1) määrittää kolmentoista suomalaisen marjakasvin lehtien ja marjojen elintarvikelaatua olevien vesi-etanoliuutteiden fenolisten yhdisteiden profiilit; 2) arvioida uutteiden antioksidanttiaktiivisuutta ja antimikrobivaikutusta elintarvikepatogeeneihin; 3) tutkia 21:n *Ribes nigrum* -genotyypin (lajikkeen) ja vuosivaihtelun vaikutuksia marjojen fenolisiin yhdisteisiin.

Fenolisten yhdisteiden kokonaispitoisuus vesi-etanoliuutteissa oli merkitsevästi korkeampi lehdissä (54–786 mg/100 ml) kuin vastaavissa marjoissa (8–71 mg/100 ml). Tyrnin lehdissä kokonaispitoisuus (606–786 mg/100 ml) oli kaikista näytteistä korkein johtuen ellagitanniinien runsaudesta. Puolukan lehtiuutteessa *β*-*p*-arbutiini kattoi yli 40 % fenolisista yhdisteistä (271 mg/100 ml) ja seuraavaksi runsaimpia olivat (+)-katekiini, prosyanidiinit ja kversetiiniglykosidit. Mustikan lehtiuutteessa oli paljon kahvihappoa, joka on esteröitynyt kiinihapon kanssa (80 % fenolisten yhdisteiden kokonaismäärästä). Antosyaniinit dominoivat tummakuorisissa marjoissa, kun taas tyrnimarjat sisälsivät ensisijaisesti isoramnetiinin glykosideja.

Uutteiden sekä antioksidatiiviset että antibakteeriset vaikutukset vaihtelivat merkittävästi korreloiden fenolisten yhdisteiden kokonaismäärään. Flavonoidit korreloivat vahvasti Folin-Cicalteu -mittausten, happiradikaalien absorptiokapasiteetin (ORAC) ja radikaalien kanssa reagoivan antioksidanttiparametrin (TRAP) tuloksiin. Korrelaatio oli erityisen vahva antioksidatiivisen aktiivisuuden ja seuraavien fenolisten yhdisteryhmien välillä: Proantosyaniinit (proantosyanidiinidimeerit ja -trimeerit), flavan-3-olit, (+)-katekiini ja (−)-epikatekiini ja glykosyloituneet flavonolit (kversetiinit). Antosyaniinit ja fenoliset ei-flavonoidiyhdisteet korreloivat voimakkaasti DPPH -radikaaleja sammuttavan aktiivisuuden kanssa. Juuri nämä ei-flavonoidiyhdisteet olivat tehokkaita inhiboimaan eräiden bakteerilajien kasvua. Tästä hyvänä

esimerkkinä on ellagitanniinien teho inhiboida eräitä *Staphylococcus aureus* and *Bacillus cereus* -kantoja.

Kahdeksan hedelmien ja lehtien uutetta valittiin Sephadex LH-20 -kolonnin avulla tapahtuvaan fraktiointiin. Jakeiden antioksidantti- ja antimikrobisia aktiivisuuksia tutkittiin edelleen tavoitteena löytää vaikuttavimmat fenoliset yhdisteet. Tulosten mukaan kversetiiniglykosidien ORAC -aktiivisuudet alenivat yhdisteiden sokerikomponenttien lisääntyessä. Monoglykosyyli-kversetiineillä sokeriosan luonne ilmeisesti vaikuttaa peroksyyliradikaalien sammuttamisen kapasiteettiin. *Escherichia coli* osoitti parempaa resistenssiä tutkittuihin jakeisiin verrattuna *S. aureus* -kantoihin.

Mustaherukan marjojen fenolisten yhdisteiden koostumus ja pitoisuudet vaihtelivat jonkin verran lajikkeiden ja viljelyvuosien mukaan. Fenolisten happojen johdosten pitoisuudet olivat merkittävimmät erottavat tekijät Skotlannista, Liettuasta ja Suomesta peräisin olevien, mutta samassa paikassa kasvatettujen, lajikkeiden välillä. Tietyn alkuperän lajikkeet ryhmittyivät delfinidiinin ja syanidiinin 3-*O*-glykosidien perusteella. Marjat, jotka korjattiin kahtena eri vuonna, poikkesivat toisistaan fenolisten konjugaattien ja glykosyloituneiden kversetiinien perusteella.

LIST OF ABBREVIATIONS

AAPH	2'-azobis(2-amidinopropane) dihydrochloride
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
B. cereus	*Bacillus cereus*
B. subtilis	*Bacillus subtilis*
C. jejuni	*Campylobacter jejuni*
CAA	Cellular antioxidant activity
CUPRAC	Cupric reducing antioxidant capacity
DAD	Diode array detector
DHHDP	Dehydrohexahydroxydiphenic acid
DP	Degree of polymerization
DPPH	2,2-diphenyl-1-picrylhydrazyl
DQF-COSY	Double-quantum filtered-Correlation spectroscopy
DW	Dry weight
EC_{50} (or IC_{50})	Content causing 50% radicals scavenged (or inhibited)
E. coli	*Escherichia coli*
Ep/2	Half peak oxidation potentials
ESR	Electron spin resonance spectrometry
FRAP	Ferric reducing activity power
FW	Fresh weight
GAE	Gallic acid equivalent
H_2O_2	Hydrogen peroxide
HHDP	Hexahydroxydiphenic acid
HMBC	Heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum coherence
L. monocytogenes	*Listeria monocytogenes*
L. rhamnosus	*Lactobacillus rhamnosus*
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
M. luteus	*Micrococcus luteus*
MS	Mass spectrometry
MTC	Maximal tolerated concentration
NMR	Nuclear magnetic resonance
$NO^{\bullet}$	Nitric oxide radical
$OH^{\bullet}$	Hydroxyl radical
$OOH^{\bullet}$	Hydroperoxyl radical
$^{\bullet}O_2$	Singlet oxygen radical
$O_2^{\bullet-}$	Superoxide radical
$OOR^{\bullet}$	Peroxyl radical

ORAC	Oxygen radical absorbance capacity
P. aeruginosa	*Pseudomonas aeruginosa*
PCA	Principal component analysis
PLS	Partial least squares regression
P. putida	*Pseudomonas putida*
SAR	Structure-activity relationship
S. aureus	*Staphylococcus aureus*
S. enterica	*Salmonella enterica*
S. epidermidis	*Staphylococcus epidermidis*
TE	Trolox equivalent
TEM	Transmission electron microscopy
TOCSY	Total correlation spectroscopy
TRAP	Total radical trapping antioxidant parameter
Trolox	6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
TSP	Trimethylsilylpropanoic acid
UPLC	Ultra performance liquid chromatography
UV/Vis	Ultraviolet–visible spectrophotometry
Y. enterocolitica	*Yersinia enterocolitica*

LIST OF ORIGINAL PUBLICATIONS

I. Tian, Y.; Liimatainen, J.; Alanne, A. L.; Lindstedt, A.; Liu, P.; Sinkkonen, J.; Kallio, H.; Yang, B. Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. *Food Chemistry*, **2017**, *220*, 266–281.

II. Tian, Y.; Puganen, A.; Alakomi, H-L.; Uusitupa, A.; Saarela, M.; Yang, B. Antioxidative and antibacterial activities of aqueous ethanol extracts of berries, leaves, and branches of berry plants. *Food Research International*, **2018**, *106*, 291–303.

III. Tian, Y.; Liimatainen, J.; Puganen, A.; Alakomi, H-L.; Sinkkonen, J.; Yang, B. Sephadex LH-20 fractionation and bioactivities of phenolic compounds from extracts of Finnish berry plants. *Food Research International*, **2018**, *113*, 115–130.

IV. Tian, Y.; Laaksonen, O.; Haikonen, H.; Vanag, A.; Ejaz, H.; Linderborg, K.; Karhu, S.; Yang, B. Compositional diversity among blackcurrant (*Ribes nigrum*) cultivars originating from European countries. *Journal of Agricultural Food Chemistry*, **2019**, *67*, 5621−5633.

1 INTRODUCTION

Food safety is of a major interest of consumers, authorities and food manufacturers around the world. As the main threats in food industry, food deterioration and microbial contamination causes not only the waste of food materials and economic losses, but also serious illness, which leads to public health problems (Das, Islam, Marcone, Warriner, Diarra, 2017). The global scale of foodborne illness is difficult to estimate, especially for developing countries, due to lack of the system to record the foodborne illnesses (McEntire, 2013). Despite of this, the World Health Organization (WHO) estimated that 31 foodborne diseases resulted in over 600 million illnesses and 420,000 deaths worldwide in year 2010 (Havelaar et al. 2015). The foodborne diseases are increasing in the low- and middle-income countries, and most of them relate to the consumption of contaminated and spoiled foods (Grace, 2015). For developed countries, outbreaks of foodborne illness still occur. In the United States of America, the Center for Disease Control and Prevention (CDC) estimated that 48 million American people suffer from foodborne illnesses in each year, causing 128,000 hospitalizations and 3000 deaths (CDC, 2018). One eighth of Canadian people (4 million) are infected with foodborne illness annually (Thomas et al., 2013). Over 5.2 million people have been reported annually to have foodborne diseases in Australia, despite the high standard of living and food safety control (Kirk et al. 2008).

Using preservatives is an effective way to protect food components from oxidation and to prevent the growth of foodborne pathogens, extending the shelf life of food products. Nowadays, synthetic preservatives are commonly used in food industry, such as butylated hydroxyanisole (BHA, E320), butylated hydroxytoluene (BHT, E321), tert-butylhydroquinone (TBHQ, E319), and propyl gallate (PG, E310). Safety of the compounds has been evaluated, and the safe levels not causing potential toxicological risk have been defined by authorities. There has been increasing concern about the safety of the consumers in recent years. Moreover, addition of some synthetic preservatives may cause changes or loss of sensory properties of certain foods, which also reduces the consumer acceptance. All these concerns result in an increasing demand for safe and effective food preservatives from natural sources to reduce the usage of synthetic preservatives in foods. The overuse of antibiotics is another factor raising need for development of natural food preservatives. In 2014, the WHO published a report on antibacterial resistance surveillance, showing that resistance to common antibiotics had reached alarming levels among 129 member countries. For instance, the strains of *Salmonella enterica* serotype Typhimurium have multi-resistance against multiple antibiotics including ampicillin, chloramphenicol, streptomycin, sulfonamides and

tetracyclines. Clear resistances to the third-generation cephalosporins and fluoroquinolones have been observed in 85 % and 90 % of *Escherichia coli* strains, respectively, in the investigated countries. The proportion of methicillin-resistant *Staphylococcus aureus* has exceeded 20% of all the strains found in all studied regions (WHO, 2014).

Previous studies have revealed that many natural plant extracts exhibit a great potential in controlling microbial growth and reducing oxidation damages of sensitive ingredients in foods (Tiwari, Valdramidis, O'Donnell, Muthukumarappan, Bourke, Cullen, 2009; Dudonné, Vitrac, Coutière, Woillez, Mérillon, 2009; Negi, 2012). The compounds contributing to these activities mainly belong to phenolic compounds, which are mostly found in fruits, leaves and seeds. Although a wide range of plant extracts have been reported to have significant inhibitory activities against oxidations and bacteria, commercial applications of the natural food preservatives are still limited. New research in this area is urgently needed to promote the industrial development.

The literature part of the thesis reviews previous research on the composition, anti-oxidative, and anti-bacterial activities of phenolics compounds and phenolic extracts of berry plants. The factors influencing phenolic profiles and the bioactivities are also reviewed in this section. The key emphasis is on the relationship between structure and activity of phenolics.

The thesis research is focused on the qualitative and quantitative analysis of phenolic compounds in food-grade aqueous ethanolic extracts prepared from fruits and leaves of wild and cultivated Finnish berry species. The anti-oxidative activities of the phenolic extracts are investigated using multiple *in vitro* anti-oxidative assays, and the antimicrobial efficacies tested against several foodborne pathogens. Certain extracts are chosen due to their high bioactivities, and fractionated with column chromatography. For each fraction, the antioxidant and anti-bacterial activities are investigated in order to identify the key compounds for specific target bioactivities. By analyzing the compositional and bioactivity data with statistical models, the main groups and individual compounds are pinpointed based on the contribution to bioactivities studied. The impact of genotype on phenolic profiles is also investigated with blackcurrant cultivars as examples. The results of the current research provide guidance for research and development of natural food preservatives as well as food development in food industry.

2 REVIEW OF THE LITERATURE

2.1 Phenolic compounds in berry species

Phenolic compounds are secondary metabolites of plants, synthesized biologically via the shikimate and the acetate pathways. The phenolics in berry plants are generally characterized as flavonoids and non-flavonoid compounds (Puupponen-Pimiä, Nohynek, Alakomi, Oksman-Caldentey, 2005; Shahidi, Ambigaipalan, 2015). All flavonoids share a carbon skeleton of C_6-C_3-C_6, including two benzene rings (ring A and B) coupled with a three-carbon oxygenated heterocycle (ring C). Variation in the degree of oxidation and heterogeneity at C ring leads to the multiple subclasses of flavonoids, such as flavan-3-ols, isoflavones, flavanones, flavonols, and anthocyanidins (Pereira, Valentão, Pereira, Andrade, 2009). Non-flavonoids contain mainly phenolic acids and tannins. Phenolic acid moieties in berry species are usually based on hydroxybenzoic acid and hydroxycinnamic acid, commonly present in the form of esters with sugars or other acids (Daglia, 2012). Tannins are divided into proanthocyanidins and hydrolysable tannins. The former, as called condensed tannins, belongs to polymeric flavan-3-ols; the latter mainly consists of gallotannins (mostly as esterified glucoses with gallic acids) and ellagitannins (glucose esters of gallic acid and ellagic acid). Both proanthocyanidins and hydrolysable tannins exhibit a wide structural variability, which is associated with the different linkages between the monomeric units, and the degree of polymerization (Chung, Wong, Wei, Huang, Lin, 1998; Landete, 2011).

2.1.1 Phenolic profiles of berries

Although phenolic compounds exist ubiquitously in berry plants, the fruits of various berry species display a large diversity of phenolic profile.

Anthocyanins are natural pigments responsible for the blue, purple, and red colors of many berries (de Pascual-Teresa, Moreno, García-Viguera, 2010). In the fruits of cranberry (*Vaccinium macrocarpon*, American cranberry), anthocyanins are predominant among over 150 identified phenolic compounds as described previously (Pappas, Schaich, 2009). Anthocyanins in cranberry are present mainly as 3-*O*-galactosides and 3-*O*-arabinosides of cyanidin and peonidin at a total content of 14–171 mg/100 g FW. The glycosylated delphinidin, petunidin, peonidin, pelargonidin, and malvidin are identified in some cultivars of cranberry, but generally remain at low percentage of total anthocyanins (Wu et al., 2006). Blackberries (*Rubus fruticosus*) contain high levels of anthocyanins, total content of which is 114–242 mg/100 g of fresh fruits, depending on the genotype of blackberry (Cho, Howard, Prior, Clark,

2004). Cyanidin is the primary anthocyanidin in blackberries, and present as 3-*O*-glycosides with rutinose, glucose, xylose and arabinose being the major sugar moieties. In some varieties of blackberries, the sugar moieties sometimes are acylated with acetic acid, *p*-coumaric acid, and malonic acid (Wu, Beecher, Holden, Haytowitz, Gebhardt, Prior, 2006; Oszmiański, Nowicka, Teleszko, Wojdyło, Cebulak, Oklejewicz, 2015). In the crowberries (*Empetrum nigrum*), the total concentration of anthocyanins varies in the berries originating from different countries. Determined by HPLC, Canadian black crowberries contain 503–690 mg/100 g FW of anthocyanins, whereas the total content in Finnish crowberries is 5500 mg/100 g FW, and 4200 mg/100 g FW in Japanese berries. The content difference among crowberries is likely the outcome of complex interplay of multiple factors including subspecies, cultivation, climatic conditions, the stage of ripeness, harvesting time, storage conditions (Wang, Lin, 2000; Castrejón, Eichholz, Rohn, Kroh, Huyskens-Keil, 2008; Zheng, et al., 2012; Yang, Zheng, Laaksonen, Tahvonen, Kallio, 2013). Despite the large deviation in total content of anthocyanins, 3-*O*-galactosides of cyanidin, delphinidin, and malvidin are the major anthocyanins in the berries of *Empetrum nigrum* from these three countries (Bakowska-Barczak, Marianchuk, Kolodziejczyk, 2007; Dudonne et al., 2015; Laaksonen, Sandell, Järvinen, Kallio, 2011; Ogawa et al., 2008).

Flavonols are another main group of flavonoids, presenting usually as *O*-glycosides in berry species. Glucose is the major sugar residue, whereas other sugar residues such as galactose, rhamnose, arabinose, xylose and glucuronic acid are also found commonly (Häkkinen, 2000). Mikulic-Petkovsek and co-workers compared the total content of flavonols in the methanolic extracts of berries of twenty-eight species (Mikulic-Petkovsek, Slatnar, Stampar, Veberic, 2012). The HPLC data suggested that elderberries contained the highest amount of total flavonols (45–57 mg/100 g FW); whereas the lowest were found in strawberries (*Fragaria* × *ananassa*, 1 mg/100 g) and whitecurrants (*Ribes glandulosum*, 0.5 mg/100 g). Other rich sources of flavonols included the fruits of chokeberry (*Aronia melanocarpa*, 27 mg/100 g FW), wild blackberry (26 mg/100 g), rowanberry (*Sorbus aucuparia*, 23 mg/100 g), cranberry (21 mg/100 g) and blackcurrants (*Ribes nigrum*, 20 mg/100 g). For sub-groups of flavonols, quercetin glycosides were present in most of the berries studied, accounting for 46–100 % of total flavonols. Glycosides of isorhamnetin and kaempferol represented the dominant flavonols in wild strawberries and gooseberry (*Ribes grossularia*), and kaempferols were also the prevailing in currants (*Ribes* spp.). Myricetin derivatives were detected in chokeberry and rowanberry (Mikulic-Petkovsek, Slatnar, Stampar, Veberic, 2012). For other berry species, Ma *et al*. reported that sea buckthorn (*Hippophaë rhamnoides* L.) berries were also rich in flavonol glycosides, the

total content of which was up to 23 to 250 mg/100 g of fresh berries (Ma et al., 2016). The glycosides of isorhamnetin (45–78% of total flavonols) and quercetin (22–50%) form the predominant flavonoids as reported previously (Chen, Zhang, Xiao, Yong, Bai, 2007; Yang, Halttunen, Raimo, Price, Kallio, 2009). In crowberries, the content of total flavonols varies from 37 to 390 mg/100 g of fresh fruits. Quercetin glycosides are the majority, and the derivatives of morin, kaempferol, and myricetin are identified only in certain cultivars (Jurikova et al., 2016).

Proanthocyanidins and ellagitannins in berries have been studied extensively. The large structural diversity of these tannins leads to great challenges in the analyses. The quantification of proanthocyanidins has been currently conducted by the degree of polymerization (DP) only, due to analytical challenges. Hawthorn (*Crataegus* spp.) fruits are well-known for containing significant amounts of proanthocyanidins. Based on the results of Liu *et al*., thirty-six of procyanidins and procyanidin derivatives are identified from the fruits of 22 cultivars of Chinese hawthorn at a total content of 250−3669 mg/100 g of dry matters (Liu, Kallio, Lü, Zhou, Yang, 2011). Hosseinian *et al*. investigated the profiles of proanthocyanidins in the fruits of six Canadian berry species, and found oligomeric and polymeric procyanidins being the majority in all berries studied. Raspberries contained the highest total-content of procyanidins (505 mg/100 g FW), followed by strawberries (447 mg/100 g), and saskatoon berries (*Amelanchier alnifolia*, 369 mg/100 g). Procyanidins remained at low levels in chokecherries (286 mg/100 g), sea buckthorn berries (276 mg/100 g), and wild blueberries (259 mg/100 g) (Hosseinian et al., 2007). Yang and co-workers studied wild sea buckthorn berries (spp. *rhamnoides*, *sinensis*, and *mongolica*) of Canadian, Chinese, and Finnish origins. The total concentration of proanthocyanidins ranged from 390 to 1940 mg/100 g DW, suggesting the impact of genotype and growth location (Yang, Laaksonen, Kallio, Yang, 2016). Moreover, the content of proanthocyanidins was up to 400 mg/100 g in fresh cranberries, representing as the derivatives of dimers to decamers. However, polymers with DP > 10 form the major components of the total proanthocyanidins, although these compounds were seldom determined individually (Gu et al., 2004).

The fruits of cloudberry (*Rubus chamaemorus*), raspberry (*Rubus idaeus*), blackberry, and strawberry are the rich sources of ellagitannins. Some amounts of ellagitannins are found in sea buckthorn berries (Landete, 2011; Kaume, Howard, Devareddy, 2012; Baby, Antony, Vijayan, 2018). The total content of ellagitannins in cloudberry has been reported as approximately 312 mg/100 g of fresh berries (Koponen, Happonen, Mattila, Törrönen, 2007). Red raspberries contain 297 mg/100 g FW of ellagitannins on average (Landete, 2011). The major ellagitannins in raspberries are lambertianin C and sanguiin

H-6. The former commonly ranges from 28 to 63 mg/100 g of fresh fruits, and the latter is up to 36–75 mg/100 g FW. The ratio of lambertianin C and sanguiin H-6 (0.8–1.2) is dependent of cultivars, so is the deviation on content of each of these compounds. (Gasperotti, Masuero, Vrhovsek, Guella, Mattivi, 2010; Klewicka, Sójka, Klewicki, Kołodziejczyk, Lipińska, Nowak, 2016). In blackberries, the total amount of ellagitannins is 85–131 mg/100 g FW, depending on cultivars (Gasperotti, Masuero, Vrhovsek, Guella, Mattivi, 2010). Sanguiin H-6 and lambertianin C are also the dominant compounds, accounting for over 50% of total ellagitannins in the fruits of blackberry cultivars (Hager, Howard, Liyanage, Lay, Prior, 2008; Gasperotti, Masuero, Vrhovsek, Guella, Mattivi, 2010). As in cloudberry, raspberry and blackberry, ellagitannins are also abundant in strawberries. The major constituents of ellagitannin present in strawberries are galloyl-bis-HHDP-glucose moieties and ellagic acid glycosides (Seeram et al., 2006). Koponen *et al*. reported that the fruits of strawberry contained ellagitannins at a total content of 75 mg/100 g FW. This is in agreement with the study of Giampieri and co-workers where the total content of ellagitannin is 25–59 mg/ 100 g of fresh strawberries (Koponen, Happonen, Mattila, Törrönen, 2007; Giampieri, Tulipani, Alvarez-Suarez, Quiles, Mezzetti, Battino, 2012).

Phenolic acids, as another major group of non-flavonoid phenolic compounds, are present commonly in berry fruits. In the study of Zuo and co-workers, fifteen phenolic acids were characterized from berry extracts of American cranberry, and benzoic acid and its derivatives were the most abundant ones (Zuo, Wang, Zhan, 2002). The pool of phenolic acids in the fruits of crowberry consists of hydroxycinnamic acid, hydroxybenzoic acid, and their derivatives. Dudonne suggested that the total content of phenolic acids was approximately 12 mg/100 g FW, and *p*-coumaric acid represented 68% of the total phenolic acids, followed by *m*-coumaric acid and coumaric acid glucoside (Dudonne et al., 2015). Caffeic acid, ferulic acid, *p*-hydroxybenzoic acid, gallic acid, and protocatechuic acids are detected at low levels (Ogawa et al., 2008; Laaksonen, Sandell, Järvinen, Kallio, 2011).

2.1.2 Phenolic profiles of leaves

As the major byproducts of berry cultivation, the leaves of berry plants are also rich sources of phenolic compounds. Compared to berries, leaves represent a distinguishing phytochemical composition.

In general, the concentration of anthocyanins is the main compositional difference of phenolics between berries and leaves of one and the same plant. Responsible for the color, anthocyanins are normally present at high concentration in the fruits of the berry plants. Vagiri *et al*. identified the phenolic compounds from buds, leaves, and fruits of Swedish blackcurrants

(*Ribes nigrum*). The result suggested that 3-*O*-rutinosides and 3-*O*-glucosides of both delphinidin and cyanidin were the primary compounds in berries, but were presented in leaves only at trace amounts (Vagiri, Ekholm, Andersson, Johansson, Rumpunen, 2012). The leaves of blackcurrant mainly contain flavonol glycosides and hydroxycinnamic acids. Quercetin, kaempferol and myricetin form the majority of the flavonol compounds (Vagiri et al., 2015). The sugar moiety of flavonol derivatives is commonly acylated with malonic acid. Altogether, twelve malonylglycosides of flavonols have been detected in the leaves of different cultivars of blackcurrant (Liu, Kallio, Yang, 2014). The dominant ones were quercetin 3-*O*-malonylglucoside (243–361 mg/100 g DW), an isomer of kaempferol 3-*O*-malonylglucoside (94–139 mg/100 g DW), and kaempferol 3-*O*-malonylglucoside (20–28 mg/100 g DW) (Vagiri et al., 2015). Other flavonol glycosides, such as 3-*O*-rutinoside and 3-*O*-glucoside of isorhamnetin, are also present in the ethanolic extract of blackcurrant leaves (Vagiri, Ekholm, Andersson, Johansson, Rumpunen, 2012). Many factors affect the flavonol profiles in the leaves of blackcurrants. The content of flavonols increases during the growth season; however, the time for reaching the concentration peak differs among various cultivars and years (Liu, Kallio, Yang, 2014). For certain compounds, the concentration is also influenced by the growth position of leaves (Vagiri et al., 2015). Compared with growing year and growth latitude, harvesting time and leaf position were more prominent factors affecting the composition of phenolic compounds in blackcurrant leaves (Yang, Alanne, Liu, Kallio, Yang, 2015). Hydroxycinnamic acids in the leaf are identified primarily as chlorogenic acid and neochlorogenic acid, and the esters of caffeic acid and quinic acid (Oszmiański, Wojdylo, Gorzelany, Kapusta, 2011; Vagiri, Ekholm, Andersson, Johansson, Rumpunen, 2012). Furthermore, several proanthocyanidins (mainly as oligomeric gallocatechins or epigallocatechins) have been recorded in leaves of *Ribes nigrum* (Tits, Angenot, Poukens, Warin, Dierckxsens, 1992; Liu, Kallio, Yang, 2014).

Wu *et al*. studied leaf extracts of 104 blueberry (*Vaccinium* ssp.) cultivars, and found that caffeoylquinic acids were the most abundant compounds, representing mainly as 5-*O*-, and 3-*O*-caffeoylquinic acid, and other isomers (Wang et al., 2015). The average total content of caffeoylquinic acids was shown as high as 3326 mg/100 g in dry leaves, but it may have a deviation among different taxa of the genus *Vaccinium* (Wang et al., 2015). According to the report of Ferlemi *et al*., the leaves of northern highbush blueberry (*V. corymbosum*) contained 6934 mg/100 g DW of caffeoylquinic acids (Ferlemi et al., 2015). Nevertheless, Harris *et al*. found that the total content of caffeoylquinic acids was 3119 mg/100 g DW in the leaves of *V. angustifolium*, which was twenty-fold compared with the corresponding berries (Harris et al.,

2007). As the second most abundant class of phenolics in blueberry leaves, the content of flavonols ranged from 551 to 3389 mg/100 g of dry matters in total. Compared to glycosides of myricetins and kaempferol, quercetin derivatives account for 68-85% of total content of flavonols (Wang et al., 2015). The profiles of quercetin derivatives were associated strongly with the genotype of cultivars, 3-*O*-galactoside, glucoside, and arabinoside being generally the dominant (Harris et al., 2007; Wang et al., 2015). Proanthocyanidins in blueberry leaves were mainly procyanidins (34–838 mg/100 g DW), including both A- and B-type compounds (Wang et al., 2015). A small amount of anthocyanins was quantified in majority of the blueberry leaves, primarily as cyanidin derivatives. For some cultivars, the total concentration of anthocyanins in leaves was ten times less than that in fruits (Virachnee, Mary, George, John, 2008; Wang et al., 2015).

2.2 Anti-oxidative activity of extracts of berry species

Many previous studies have confirmed that the extracts of berry species exhibit a powerful capacity against various free radicals, which is attributed to the presence of phenolic compounds (**Table 1**). The antioxidant activity of various berry species is dependent on the nature of free radicals. Wang and Jiao evaluated the radical scavenging ability of fruit juices of blackberry, blueberry, cranberry, raspberry (*Rubus idaeus* & *Rubus occidentalis*), and strawberry (*Fragaria* × *ananassa*). Juices of both blackberry and strawberry showed the most potent inhibition against hydroxyl ($OH^{\bullet}$), singlet oxygen ($^{\bullet}O_2$), superoxide radicals ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) in the *in vitro* assays. A weak efficacy was observed in cranberry against hydrogen peroxide, and blueberry was low in inhibiting radicals of $OH^{\bullet}$ and $^{\bullet}O_2$ (Wang, Jiao, 2000). Similar results were obtained in the study of de Souza *et al*. utilizing 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (de Souza, Pereira, da Silva, de Oliveira Lima, Pio, Queiroz, 2014). In this study, five Brazilian berries were ranked by decreasing ABTS activity as the order: blackberry > cherry > strawberry > red raspberry > blueberry. For $DPPH^{\bullet}$ radicals, blackberry and strawberry were superior to red raspberry (*Rubus idaeus*), cherry (*Prunus* spp.), and blueberry. Both ABTS and DPPH results correlated strongly to the total content of phenolics (coefficient values was 0.83 and 0.91, respectively). The activity measured by ABTS assay was contributed mostly by monomeric anthocyanins, whereas total content of flavonoids correlated mainly to DPPH assay (de Souza, Pereira, da Silva, de Oliveira Lima, Pio, Queiroz, 2014).

Ogawa *et al*. investigated the profiles of anthocyanins in ten different berry species and evaluated their antioxidant activities by using ABTS, DPPH, and

ferric reducing activity power (FRAP) assays. The acidic methanol extract of crowberry (*Empetrum nigrum*) showed strong antioxidant capacity (90% of $DPPH^{\bullet}$ scavenged, 64 % of $ABTS^{\bullet+}$ quenched, and 317 TE mg/mL of FRAP), followed by bilberry (*Vaccinium myrtillus*), blackcurrant (*Ribes nigrum*), and redcurrant (*Ribes rubrum*). As proposed by Ogawa *et al*., this might be owing to the highest concentration of anthocyanins presented in crowberry (42 mg/g dry weight, quantified by HPLC). Nevertheless, for other berries such as blueberry and cranberry, the content of anthocyanins was not in proportion to anti-oxidative activity. Therefore, other phenolic compounds in berry extracts may also have participated in scavenging free radicals (Ogawa et al., 2008).

Zheng and Wang compared the oxygen radical absorbance capacity (ORAC) of fruit extracts of blueberry, cranberry, chokeberry (*Aronia melanocarpa*), and lingonberry (*Vaccinium vitis-idaea*) (Zheng, Wang, 2003). The results suggested that chokeberry had strong anti-oxidative capacity, which was 4 to 9 times higher than those of other berries. This may have been due to the higher abundance of phenolic compounds (26 GAE/g of fresh weight) in chokeberry extracts. The correlation coefficient indicated that ORAC activity of these four berries was likely contributed by the content of total phenolics ($R^2 = 0.998$) and total anthocyanins ($R^2 = 0.951$).

In order to determine the contribution of individual phenolics, the main compounds in each sample were characterized and fractionated before ORAC analysis. Cyanidin 3-*O*-glycosides, primarily arabinosides, galactosides, and xylosides, contributed half of the ORAC activity of the extract of chokeberry, followed by caffeic acid and its derivatives (41 %). Cyanidin 3-*O*-galacotside was the major contributor in lingonberry extract, representing 44% of anti-oxidative capacity. Chlorogenic acid and glycosylated peonidins were mainly responsible for the inhibitory effects of blueberry and cranberry, respectively, against peroxyl radicals. For lingonberry, the sum of ORAC value of individual compounds was remarkably lower than that of the raw extract (13 vs. 38 μmol of TE/g). This large deviation also occurred in other berries studied, implying importance of synergy among phenolic compounds and the role of unidentified compounds scavenging peroxyl radicals (Zheng, Wang, 2003).

The anti-oxidative potency of berry species depends on both inherent structure and the concentration of phenolics. Therefore, any factor causing a change in phenolic profiles may result in the variation in antioxidant activity. The impacts of solvent and extraction time on antioxidant activity were investigated by Lapornik *et al*. using by-products of grape, blackcurrant, and redcurrant (Lapornik, Prosek, Wondra, 2005). Each berry by-product was mixed with water, 70 % aqueous ethanol, 70 % aqueous methanol; and shaken at room temperature for 1, 12, and 24 hours to obtain extracts. Determined by DPPH and β-carotene assays, the water extracts exhibited the lowest capacity

as opposed to methanol and ethanol extracts, although the difference varied among samples studied. Generally, extraction time was in proportion to the anti-oxidative values. The antioxidant activity correlated significantly to the total content of anthocyanins; however, the coefficient was lower than that shown with total phenolics.

Sharma *et al*. tested the influence of extraction procedures on antioxidant activity (Sharma, Sharma, Sharma, Sharma, Singh, Sinha, 2008). In this study, four procedures, including microwave, ultrasound, Soxhlet, and maceration, were performed to extract phenolic compounds from seeds, leaves, pulp, and fruits of sea buckthorn (*Hippophaë rhamnoides*). The anti-oxidative results of ABTS and DPPH assays suggested that microwave-assisted extracts were superior to those extracted by other approaches, which may be associated with the increased content of certain flavonols, such as quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactosides, myricetin, and isorhamnetin.

Ehlenfeldt and Prior compared leaves and fruits of eighty-seven blueberry cultivars by using ORAC measurement (Ehlenfeldt, Prior, 2001). The ORAC value ranged from 5 to 31 TE/g in fresh berries and from 245 to 971 TE/g in fresh leaves, indicating an essential role of genotype in determining the radical scavenging capacities. High ORAC activities was presented in the leaf extracts of cultivars ‘Little Giant’, ‘Darrow’, ‘Magnolia’, ‘Pearl River’, and ‘Concord’; and berry extracts of ‘Rubel’, ‘Elliott’, ‘Ornablue’, ‘Friendship’, and ‘Burlington’. There was no significant correlation in anti-peroxyl radical ability between leaf and berry extracts. The total content of phenolics had major correlation with anti-oxidative activity with coefficient values of 0.87 (p = 0.001, n = 77) and 0.76 (p = 0.001, n = 87) in leaves and berries, respectively. Anthocyanins represented a moderate correlation (R^2 = 0.57, p = 0.001, n = 87) to fruit ORAC activity. Hukkanen *et al*. studied 9 cultivars of rowanberry (*Sorbus aucuparia*) (Hukkanen, Pölönen, Kärenlampi, Kokko, 2006). The cultivars also showed a remarkable deviation in anti-oxidative activity as suggested by FRAP and DPPH assays. The extracts from cultivars ‘Rubinovaja’ and ‘Rosina’ showed the highest and lowest anti-oxidative efficacy, respectively; which corresponded to the total content of phenolics. Both FRAP and DPPH results correlated strongly with the total content of phenolics. Yet, the main contributor to antioxidant activity was not determined. As the major phenolic compounds, anthocyanins were associated moderately with FRAP assays (R^2= 0.470, p = 0.01), and no correlation was established between hydroxycinnamic acid and the measurements.

The anti-oxidative activities of berries are affected by the development stage of the plant and therefore by the sample collecting time. Wang and Lin analyzed phenolic contents and anti-oxidative potency of berries and leaves of strawberry, blackberry, and red raspberry. All the samples were collected in a

period of two weeks during fruit-bearing season. The results showed that higher ORAC activity was found in ripe fruits of red raspberry, and green-skinned fruits of both blackberry and strawberry. Linear correlations were established between ORAC activity and total phenolics for both leaves and berries, and between ORAC activity and total anthocyanins among the ripe berries. This suggested that the main contributors to peroxyl-radicals scavenging activities varied according to the stage of fruit maturity (Wang, Lin, 2000). The effect of maturity was also observed in the study of lingonberry (Wang, Feng, Bowman, Penhallegon, Ding, Lu, 2005). The total content of phenolic compounds correlated significantly to ORAC activity when lingonberry fruits were at green (R^2 = 0.9047), pink (R^2 = 0.8462), and ripe stages (R^2 = 0.9026). The total content of anthocyanins exhibited an increasing correlation with ORAC activity between pink-skinned (R^2 = 0.7215) and ripen fruits (R^2 = 0.8029); however, no correlation was found in the berries at their green stage. In contrast, $DPPH^{\bullet}$ scavenging capacity of lingonberry was in decreasing order: green fruits > pink fruits > ripe fruits (Wang, Feng, Bowman, Penhallegon, Ding, Lu, 2005).

Piljac-Žegarac and Šamec monitored the change of antioxidant capacities of strawberry, raspberry and redcurrant during storage at 4 °C before becoming spoiled visually (Piljac-Žegarac, Šamec, 2011). The anti-oxidative activities showed large fluctuations as indicated by ABTS, DPPH, and FRAP assays, being higher at the end than the beginning of storage. This may be ascribed to the slightly increased total content of phenolic compounds (primarily anthocyanins and flavonols). The stability of phenolic compounds during storage was investigated in the study of Mäkilä *et al*. (Mäkilä, Laaksonen, Alanne, Kortesniemi, Kallio, Yang, 2016). Based on the observation in mass spectrometric analysis, anthocyanins and flavonol glycosides degrade during storage at 4 °C, releasing the corresponding aglycones. Since the aglycones have been confirmed as more potent antioxidants than their glycosylated derivatives, this might explain the increase of antioxidant activity during storage time observed in the related studies.

Table 1. Previous studies on *in vitro* anti-oxidative activity of berries of various species

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Antioxidant assay*	*Literature*
bilberry	*Vaccinium myrtillus*	leaf stem	total phenolics [a] phenolic acids [c] flavonoids [c] tannins [c]	DPPH	Bujor, Le, Bourvellec, Volf, Popa, Dufour (2016)
	Vaccinium myrtillus	fruit	total phenolics [a] total anthocyanins [b] phenolic acids [c] flavonoids [c]	ABTS FRAP	Garzón, Narváez, Riedl, Schwartz, (2010)
	Vaccinium myrtillus	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
	Vaccinium myrtillus	fruit	total phenolics [a] phenolic acids [f] flavonoids [f]	ABTS	Ehala, Vaher, Kaljurand, (2005)
blackberry	*Rubus* spp.	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	ABTS DPPH *β*-carotene	de Souza, Pereira, da Silva, de Oliveira Lima, Pio, Queiroz, (2014)
	Rubus fruticosus	fruit	anthocyanins [c]	DPPH, ABTS, FRAP	Ogawa, et al., (2008)
	Rubus spp. (3 cultivars)	fruit leaf	total phenolics [a] total anthocyanins [b]	ORAC	Wang, Lin, (2000)
	Rubus spp.	fruit	-	$O_2^{\bullet-}$ scavenging H_2O_2 scavenging $OH^{\bullet}$ scavenging $^{\bullet}O_2$ scavenging	Wang, Jiao, (2000)
blackcurrant	*Ribes nigrum* spp.	fruit	total phenolics [a] total anthocyanins [b] anthocyanins [c]	ABTS FRAP	Bustos, Rocha-Parra, Sampedro, de Pascual-Teresa, León, (2018)

(**Table 1** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Antioxidant assay*	*Literature*
	Ribes nigrum	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
	Ribes nigrum var. 'Rosenthal Falch'	marc	total phenolics [a] total anthocyanins [b] anthocyanins [c]	DPPH *β*-carotene	Lapornik, Prosek, Wondra, (2005)
	Ribes nigrum	fruit	total phenolics [a] phenolic acids [f] flavonoids [f]	ABTS	Ehala, Vaher, Kaljurand, (2005)
blueberry	*Vaccinium corymbosum*	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	ABTS DPPH *β*-carotene	de Souza, Pereira, da Silva, de Oliveira Lima, Pio, Queiroz, (2014)
	Vaccinium formosum	leaf	total phenolics [a] phenolic acids [c] flavonols [c]	DPPH reducing power ORAC	Deng, et al., (2014)
	Vaccinium corymbosum var. "Nelson", "Elliot"	leaf	total phenolics [a] total anthocyanins [b]	DPPH FRAP	Routray, Orsat, (2014)
	Vaccinium corymbosum (4 cultivars)	fruit	total phenolics [a] phenolic acids [c] flavonols [c]	ABTS ESR	Castrejón, Eichholz, Rohn, Kroh, Huyskens-Keil, (2008)
	Vaccinium spp.	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
	Vaccinium corymbosum cv. 'Sierra'	fruit	total phenolics [a] total anthocyanins [b] phenolic acids [c] flavonoids [c]	ORAC	Zheng, Wang, (2003)
	Vaccinium corymbosum (87 cultivars)	fruit leaf	total phenolics [a] total anthocyanins [b]	ORAC	Ehlenfeldt, Prior, (2001)

(**Table 1** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Antioxidant assay*	*Literature*
	Vaccinium spp.	fruit	-	$O_2^{\bullet-}$ scavenging H_2O_2 scavenging $OH^{\bullet}$ scavenging $^{\bullet}O_2$ scavenging	Wang, Jiao, (2000)
chokeberry	*Aronia melanocarpa* cv. 'Elliott'	fruit	total phenolics [a] total anthocyanins [b]	ABTS FRAP	Samoticha, Wojdyło, Lech, (2016)
	Aronia mitschurinii cv. 'Viking'	juice	total phenolics [a] phenolic acids [c] flavonoids [c]	DPPH FRAP	Bolling, et al. (2015)
	Aronia melanocarpa	fruit	total phenolics [a] total anthocyanins [b] phenolic acids [c] flavonoids [c]	ORAC	Zheng, Wang, (2003)
cranberry	*Vaccinium oxycoccus*	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
	Vaccinium oxycoccus	fruit	total phenolics [a] phenolic acids [f] flavonoids [f]	ABTS	Ehala, Vaher, Kaljurand, (2005)
	Vaccinium macrocarpon cv. 'Ben Lear'	fruit	total phenolics [a] total anthocyanins [b] phenolic acids [c] flavonoids [c]	ORAC	Zheng, Wang, (2003)
	Vaccinium macrocarpon	fruit	-	$O_2^{\bullet-}$ scavenging H_2O_2 scavenging $OH^{\bullet}$ scavenging $^{\bullet}O_2$ scavenging	Wang, Jiao, (2000)
crowberry	*Empetrum nigrum*	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)

(**Table 1** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Antioxidant assay*	*Literature*
elderberry	*Sambucus nigra.*	fruit leaf	total phenolics [a] total flavonoids [e] phenolic acids [c] flavonoids [c]	ABTS DPPH NO• scavenging $O_2^{\bullet-}$ scavenging	Pinto, Spínola, Llorent-Martínez, Fernández-de Córdova, Molina-García, Castilho, (2017)
	Sambucus nigra	fruit branch	total phenolics [a] total anthocyanins [b] anthocyanins [c] flavonols [c]	ABTS OH• scavenging NO• scavenging	Silva, Ferreira, Nunes, (2017)
hawthorn	*Crataegus pinnatifida*	fruit	total phenolics [a] total flavonoids [d] phenolic acids [c] flavonols [c] tannins [c]	ORAC OOR• scavenging CAA	Wen, Guo, Liu, You, Abbasi, Fu, (2015)
	Crataegus oxyacantha	fruit	procyanidins [c]	OH• scavenging $O_2^{\bullet-}$ scavenging	Liu, Cao, Zhao, (2010)
lingonberry	*Vaccinium vitis-idaea*	fruit leaf stem	total phenolics [a] phenolic acids [c] flavonoids [c]	DPPH	Bujor, Ginies, Popa, Dufour, (2018)
	Vaccinium vitis-idaea	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	DPPH CUPRAC	Dróżdż, Šėžienė, Wójcik, Pyrzyńska, (2017)
	Vaccinium vitis-idaea	fruit	total phenolics [a] phenolic acids [f] flavonoids [f]	ABTS	Ehala, Vaher, Kaljurand, (2005)
	Vaccinium vitis-idaea (13 cultivars)	fruit	total phenolics [a] total anthocyanins [b]	ORAC	Wang, Feng, Bowman, Penhallegon, Ding, Lu, (2005)
	Vaccinium vitis-idaea cv. ‘Amberland’	fruit	total phenolics [a] total anthocyanins [b] phenolic acids [c] flavonoids [c]	ORAC	Zheng, Wang, (2003)

(**Table 1** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Antioxidant assay*	*Literature*
mulberry	*Morus nigra*	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
raspberry	*Rubus idaeus* cv. 'Autumn Blis'	fruit	total phenolics [a] total anthocyanins [b] anthocyanins [c]	ABTS FRAP	Bustos, Rocha-Parra, Sampedro, de Pascual-Teresa, León, (2018)
	Rubus idaeus	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	ABTS DPPH β-carotene	de Souza, Pereira, da Silva, de Oliveira Lima, Pio, Queiroz, (2014)
	Rubus idaeus	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	DPPH ABTS FRAP	Piljac-Žegarac, Šamec, (2011)
	Rubus idaeus	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
	Rubus occidentalis cv. 'Jewel'	fruit leaf	total phenolics [a] total anthocyanins [b]	ORAC	Wang, Lin, (2000)
	Rubus idaeus (4 cultivars)	fruit leaf	total phenolics [a] total anthocyanins [b]	ORAC	Wang, Lin, (2000)
	Rubus idaeus *Rubus occidentalis*	fruit	-	$O_2^{\bullet-}$ scavenging H_2O_2 scavenging $OH^{\bullet}$ scavenging $^{\bullet}O_2$ scavenging	Wang, Jiao, (2000)
redcurrant	*Ribes rubrum* spp.	fruit	total phenolics [a] total anthocyanins [b] anthocyanins [c]	ABTS FRAP	Bustos, Rocha-Parra, Sampedro, de Pascual-Teresa, León, (2018)
	Ribes rubrum	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	DPPH ABTS FRAP	Piljac-Žegarac, Šamec, (2011)

(**Table 1** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Antioxidant assay*	*Literature*
	Ribes rubrum	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
	Ribes rubrum var. 'Rondom'	marc	total phenolics [a] total anthocyanins [b] anthocyanins [c]	DPPH *β*-carotene	Lapornik, Prosek, Wondra, (2005)
	Ribes rubrum	fruit	total phenolics [a] phenolic acids [f] flavonoids [f]	ABTS	Ehala, Vaher, Kaljurand, (2005)
rowanberry	*Sorbus aucuparia* (9 cultivars)	fruit	total phenolics [a] phenolic acids [c] flavonoids [c]	DPPH FRAP	Hukkanen, Pölönen, Kärenlampi, Kokko, (2006)
saskatoon berry	*Amelanchier alnifolia* (4 cultivars)	fruit	phenolic acids [c] flavonoids [c] tannins [c]	ABTS FRAP	Lachowicz, Oszmiański, Pluta, (2017)
sea buckthorn	*Hippophaë rhamnoides* cv. 'Sinensis'	fruit	total phenolics [a] phenolic acids [c] flavonoids [c]	ORAC OOR• scavenging CAA	Guo, et al. (2017)
	Hippophaë rhamnoides (4 cultivars)	fruit	total phenolics [a] total flavonoids [d] phenolic acids [c] flavonoids [c]	ORAC OOR• scavenging CAA	Guo, Guo, Li, Fu, Liu, (2017)
	Hippophaë rhamnoides	leaf	total phenolics [a] total flavonoids [e]	DPPH ABTS FRAP	Upadhyay, Kumar, Gupta, (2017)
	Hippophaë rhamnoides	fruit	total phenolics [a]	DPPH FRAP	Korekar, Dolkar, Singh, Srivastava, Stobdan, (2014)
	Hippophaë rhamnoides	leaf	total phenolics [a] total flavonoids [e] flavonols [c]	DPPH reducing power FRAP	Kumar, Dutta, Prasad, Misra, (2011)

(**Table 1** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Antioxidant assay*	*Literature*
strawberry	*Fragaria* × *ananassa* cv. 'Oso Grande'	juice	total phenolics [a] anthocyanins [c]	ABTS DPPH	Arend, et al. (2017)
	Fragaria × *ananassa*	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	ABTS DPPH *β*-carotene	de Souza, Pereira, da Silva, de Oliveira Lima, Pio, Queiroz, (2014)
	Fragaria × *ananassa*	fruit	total phenolics [a] total anthocyanins [b] total flavonoids [e]	DPPH ABTS FRAP	Piljac-Žegarac, Šamec, (2011)
	Fragaria × *ananassa*	fruit	anthocyanins [c]	DPPH ABTS FRAP	Ogawa, et al., (2008)
	Fragaria × *ananassa*	fruit	total phenolics [a] phenolic acids [f] flavonoids [f]	ABTS	Ehala, Vaher, Kaljurand, (2005)
	Fragaria × *ananassa* (8 cultivars)	fruit leaf	total phenolics [a] total anthocyanins [b]	ORAC	Wang, Lin, (2000)
	Fragaria × *ananassa*	fruit	-	$O_2^{\bullet-}$ scavenging H_2O_2 scavenging $OH^{\bullet}$ scavenging $^{\bullet}O_2$ scavenging	Wang, Jiao, (2000)

[a] total content of phenolics was measured by Folin−Ciocalteu assays; [b] total content of anthocyanins was measured by pH differential methods; [c] individual phenolic compounds were quantified by HPLC; [d] total content of flavonoids were determined by sodium borohydride/chloranil-based assay; [e] total content of flavonoids was determined by aluminium complex; [f] individual phenolic compounds were determined by capillary electrophoresis analyses.

2.3 Structure-antioxidant activity relationship of phenolic compounds

Phenolic compounds are known as potent antioxidants. Their anti-oxidative activities depend on the ability of scavenging free radicals, donating hydrogen atoms, transferring unpaired electron, and chelating metal cations (Balasundram, Sundram, Samman, 2006; Heim, Tagliaferro, Bobilya, 2002). Certain phenolic compounds in vegetables and beverages, such as flavonoids, exert stronger anti-oxidative capacities than vitamin C and E (Prior, Cao, 2000). The superiority of phenolics is attributed to their inherent structure, which has been summarized as structure-activity (SAR) relationship. It is important to understand the mechanism of antioxidant actions in order to predict the anti-oxidative capacities of various phenolic compounds. Nevertheless, the relationship between *in vitro* anti-oxidative activity and chemical structure of phenolics has not been established unequivocally yet. This is due to the large number of phenolic compounds and multiple *in vitro* assays stimulating various free radicals.

2.3.1 Phenolic acids

The anti-oxidative activity of phenolic acids relies on the number of hydroxyl groups (-OH) in the phenyl ring. The orientation of an -OH group, as well as its possible methoxy substitution, are the key determinants of free radical scavenging capacities, which may vary among different groups of phenolic acids depending also on the *in vitro* assay applied.

Among hydroxybenzoic acids, gallic acid (3,4,5-trihydroxybenzoic acid) exhibit the strongest inhibition against both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals, followed by di- and monohydroxybenzoic acids (**Table 2**). The $ABTS^{\bullet+}$ scavenging capacity of isomers of dihydroxybenzoic acids decreased in the order of 3,5-dihydroxybenzoic acid > 2,3-dihydroxybenzoic acid > 2,4-dihydroxybenzoic acid (3,4-dihydroxybenzoic acid) > 2,5- dihydroxybenzoic acid (Rice-Evans, Miller, Paganga, 1996; Cai, Sun, Xing, Luo, Corke, 2006). This order was not entirely in agreement with the results from DPPH assay, where both 3,5- and 2,4-dihydroxybenzoic acids had no inhibition against $DPPH^{\bullet}$ radicals (Sroka, Cisowski, 2003). Rice-Evans *et al*. reported that *m*-hydroxybenzoic acid had higher ABTS activity (0.84 mM) than its *p*- and *o*-isomers (0.08 and 0.04 mM, respectively) (Rice-Evans, Miller, Paganga, 1996). In contrast, other studies suggested that all these three acids were equally weak in quenching $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals (Sroka, Cisowski, 2003; Cai, Sun, Xing, Luo, Corke, 2006). For 4-hydroxybenzoic acid, the anti-oxidative efficacy was increased by introducing one or two methoxy groups at *ortho*-position of -OH group, such as vanillic acid, (4-OH and 3-OCH_3) and syringic

acid, (4-OH and 3,5-di OCH_3) (Dewick, 2002; Fukumoto, Mazza, 2000). Enhanced effects of vanillic acid and syringic acid were observed on scavenging DPPH (only syringic acid), $ABTS^{\bullet+}$, $O_2^{\bullet-}$, and $OH^{\bullet}$ (Zhou, Yin, Yu, 2006). The carboxylate group (-COOH) in phenyl ring is acknowledged as having electron-withdrawing property, interfering the hydrogen-donating ability of hydroxybenzoic acids. Reis and coworkers suggested that esterification of -COOH group increased DPPH-quenching ability of hydroxybenzoic acids based on the evaluation of the activities of protocatechuic acid and its alkyl esters. Their results also indicated that the increasing length of alkyl chain led to the electron-donating enhancement (Reis, et al. 2010).

The negative impact of -COOH group on radical scavenging capacity is counteracted by inserting an alkyl group (such as $-CH_2-$) or an ethylenic group (-CH=CH-) between -COOH group and phenyl ring. It may explain why most of the hydroxyphenylacetic acids and hydroxycinnamic acids are more effective radical scavengers than their benzoate counterparts (**Table 2**). The comparison between hydroxyphenylacetic and hydroxycinnamic acids has not been investigated clearly yet. Mono-hydroxyl group in hydroxycinnamic acids is stronger hydrogen donor than that in hydroxyphenylacetic acids (*o*-/*m*-/*p*-coumaric acid vs. *o*-/*m*-/*p*-hydroxyphenylacetic acid; ferulic acid vs. 4-hydroxy-3-methoxyphenylacetic acid); whereas 3,4-dihydroxyphenylacetic acid had better capacity of quenching $ABTS^{\bullet+}$ than caffeic acid (3,4-dihydroxycinnamic acid).

Among hydroxycinnamic acids, *p*-coumaric acid is a potent antioxidant in both ABTS and DPPH assays compared to *o*- and *m*-coumaric acids. The antioxidant efficiency of *p*-coumaric acid is influenced significantly by the substitution at *meta*-position related to -CH=CH-COOH group, although the results from previous studies showed some contradiction. In **Table 2**, *p*-coumaric acid (4-OH) represented higher activity against $ABTS^{\bullet+}$ than ferulic acid (4-OH, 3-OCH_3) and caffeic acid (3,4-di OH) when defined as Trolox equivalents (mM) (Rice-Evans, Miller, Paganga, 1996; Cai, Sun, Xing, Luo, Corke, 2006; Zhou, Yin, Yu, 2006). This is consistent with the study on the protective effects of hydroxycinnamates against autoxidation of fats, in increasing order of effectiveness: caffeic < ferulic < *p*-coumaric acid (Shahidi, Wanasundara, 1992). In contrast, Piazzon *et al*. found anti-oxidative activity decreased in the following order: ferulic acid > *p*-coumaric acid > sinapic acid (4-OH, 3,5-di OCH_3) > caffeic acid by comparing the slope of dose-activity curve in ABTS assay (Piazzon, Vrhovsek, Masuero, Mattivi, Mandoj, Nardini, 2012). Regarding DPPH radicals, caffeic acid inhibited 50 % of free radicals at the lowest concentration, whereas *p*-coumaric acid was ineffective as measured by either inhibition percentage (%) or concentration causing 50% radicals

scavenged (EC_{50}) (Zhou, Yin, Yu, 2006; Garcia-Parrilla, Villano, Fernandez-Pachon, Moya, Troncoso, 2007; Abramovic, Terpinc, 2010). *p*-Coumaric acid was also confirmed to be less active towards peroxyl radicals than its *meta*-substituted derivatives (Natella, Nardini, Di Felice, Scaccini, 1999). Like hydroxybenzoic acids, the anti-radical potency of hydroxycinnamic acids was also influenced by esterification on the carboxyl group. The esterification of caffeic acid resulted in an increase in its inhibition efficacy against $DPPH^{\bullet}$ radicals (Silva, Borges, Guimarães, Lima, Matos, Reis, 2000). The same enhancement to $DPPH^{\bullet}$ radical scavenging was also observed when caffeic acid was esterified with quinic acid as chlorogenic acid (Abramovic, Terpinc, 2010; Sroka, Cisowski, 2003). Yet, chlorogenic acid had equal or even lower anti-$ABTS^{\bullet+}$ potency than caffeic acid (Rice-Evans, Miller, Paganga, 1996; Piazzon, Vrhovsek, Masuero, Mattivi, Mandoj, Nardini, 2012). By occupying hydroxyl groups, glycosylation in the phenyl ring causes a markedly decrease on antioxidant activity of hydroxycinnamic acids. Both caffeic acid and ferulic acid showed stronger anti-$ABTS^{\bullet+}$ capacity compared to the corresponding sulfates and glucuronides (Piazzon, Vrhovsek, Masuero, Mattivi, Mandoj, Nardini, 2012).

2.3.2 Flavonoids

As the radical scavenger, the capacity of flavonoids is highly associated with the numbers and configuration of hydroxyl group in the molecule. The backbone of flavonoids (without a single hydroxyl group) has no contribution to quenching free radicals as described previously (Cai, Sun, Xing, Luo, Corke, 2006). The substitution at either hydroxyl group or carbon atom affects the radical scavenging capacity significantly. Presence of the structure of *ortho*-3',4'-dihydroxyl group (catechol group) in the B ring enhances the radical quenching capacity of flavonoids. This is also the case for 3-OH group in the C ring as well as 2,3-double bond combined with 4-oxo group in the C ring (Pietta, 2000; Procházková, Boušová, Wilhelmová, 2011).

The *ortho*-3',4'-dihydroxyl group in B ring is the main structural feature of flavonoids for scavenging free radicals. Besides donating hydrogen atoms, the catechol group enhances the anti-oxidative activity of flavonoids by stabilizing the flavonoid phenoxyl radicals *via* electron delocalization (Sekher Pannala, Chan, O'Brien, Rice-Evans, 2001). As shown in **Table 2**, the aglycone of quercetin exceeded kaempferol (4'-monohydroxyl group) as scavenger of both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals. Due to the absence of catechol group, the anti-radicals activity of apigenin was lower than luteolin; the same applies to pelargonidin compared to cyanidin. This structural feature contributed strongly to the inhibition of peroxyl, superoxide, and peroxynitrite radicals (Cao, Sofic, Prior, 1997; Hu et al. 1995; Haenen, Paquay, Korthouwer, Bast, 1997). The

superiority of catechol moiety was also found in chelating trace metal cations. Quercetin and luteolin were more active than their counterparts lacking of 3',4'-catechol group (Brown, Khodr, Hider, Rice-Evans, 1998). Substitution at catechol group of B ring, such as methylation, results in a steric obstruction that reduces antioxidant activity of flavonoids as reported previously (Heim, Tagliaferro, Bobilya, 2002; Dugas, Castañeda-Acosta, Bonin, Price, Fischer, Winston, 2000; Cao, Sofic, Prior, 1997).

Compared to catechol group, introducing another hydroxyl group in B ring as 3',4',5'-trihydroxyl (pyrogallol) group might not constantly increase the ability of scavenging radicals. Measured by the assays of ABTS and DPPH, epigallocatechin and quercetin (both containing pyrogallol group) showed higher Trolox equivalent (TE) values than epicatechin and myricetin, respectively, but the latter compounds were able to quench 50% free radicals at lower contents (IC_{50}). The anti-$ABTS^{\bullet+}$ capacity of anthocyanidins showed no significant influence from the third hydroxyl group when comparing cyanidin with delphinidin (**Table 2**). Taubert *et al*. reported that 3',4',5'-trihydroxyphenyl group contributed to a marked increase in $O_2^{\bullet-}$ scavenging kinetics, although myricetin had lower IC_{50} values than quercetin (Taubert et al. 2003). The pyrogallol group influenced the inhibitory activity of flavonoids differently on lipid peroxidation. Enhanced activities were observed when comparing delphinidins with cyanidins; however, (epi)gallocatechin exerted a lower capacity than (epi)catehcin (Seeram, Nair, 2002). Ratty and Das suggested that 3',4',5'-trihydroxyphenyl group increased the efficiency of inhibiting lipid oxidation, when comparing quercetin with myricetin (Ratty, Das, 1988). Nevertheless, myricetin was found to have stronger half peak oxidation potential (Ep/2) than quercetin, but lower IC_{50} (van Acker et al. 1996a). It is possible that the compounds with a 3',4',5'-trihydroxyl moiety (either in B-ring or in A-ring) exhibit anti-oxidative or pro-oxidative activities in different assays (Ohshima, Yoshie, Auriol, Gilibert, 1998). As a pro-oxidant, the flavonoid phenoxyl-radicals counteracted the anti-oxidative effect by interacting with oxygen, and producing quinones and superoxide anion instead of donating hydrogen atoms (Amić, Davidović-Amić, Beslo, Rastija, Lucić, Trinajstić, 2007).

van Acker and co-workers reported that the torsion angle of B ring relative to the rest of molecule contributes mainly to radical-scavenging of flavonoids. As an essential structural element, C_3 hydroxyl group in C ring ensures the whole molecule of flavonoids on a same plane, which permits electron delocalization and stabilization of flavonoid phenoxyl radicals (van Acker et al. 1996b). With same structural features in A- and B-rings, flavones are known to be weaker radical scavengers than flavonols (**Table 2**), since the absence of C_3-OH group in flavones resulted in 20° of torsion angle of B ring (Cody, Luft,

1994). In contrast, esterification at C_3-OH group with a gallic acid enhanced the antioxidant capacity of flavan-3-ols (**Table 2**). The structure of flavan-3-ol gallates is a saturated heterocycle, which means no electron delocalization between A- and B-ring. Thus, the number of hydroxyl group is mainly responsible for the ability of quenching radicals (Rice-Evans, Miller, Paganga, 1996).

For flavonoids with unsaturated C ring, the anti-radical activity is reduced by blocking C_3-OH group *via* methylation or glycosylation. The anti-oxidative activities of some 3-*O*-glycosylated flavonols and anthocyanidins are given in **Table 2**. Due to the loss of co-planarity; both TE and IC_{50} value of kaempferol, quercetin, cyanidin, and malvidin were reduced remarkably after C_3-OH group substituted by glycosyl groups. This is consistent with the study of Haenen et al., reporting that IC_{50} of quercetin 3-*O*-rutinoside was three times higher than its aglycone for inhibiting peroxynitrite radicals (Haenen, Paquay, Korthouwer, Bast, 1997). Glycosylated flavonols and anthocyanidins showed lower efficiency for scavenging superoxide radical compared to their aglycones (Sichel, Corsaro, Scalia, Di Bilio, Bonomo, 1991). For radical-scavenging ability, the negative influence of glycosyl group at C_3 position is dependent on the number of sugar moieties. Plumb et al. extracted the glycosides of kaempferol and quercetin from various tea (Lapsang souchong, Assam, Darjeeling, Keemun, Ceylon and Nunjo) leaves, and found that tri-glycosides of kaempferol and quercetin were less effective antioxidants than the corresponding monoglycosides and diglycosides (Plumb et al. 1999). The increased numbers of sugar moieties at C_3 attenuated the inhibition potency of cyanidins against lipid peroxidation (Seeram, Nair, 2002). No clear pattern has been shown on the impact of the structure of sugar moiety on the anti-oxidative activities of flavonol glycosides in the studies reported so far (**Table 2**). Besides glycosylation, methylation at C_3-OH group may also decrease the inhibitory activity of flavonoids on oxidation of *β*-carotene in linoleic acid system (Burda, Oleszek, 2001).

Although not participating in hydrogen-transferring, the C_2-C_3 double bond in conjugation with the C_4-oxo group of C ring is essential for a potent inhibitory capacity against free radicals. This arrangement connects the A- and B-ring, and stabilizes the flavonoid phenoxyl radicals with a resonance effect of the aromatic nucleus (Bors, Heller, Michel, Saran, 1990). **Table 2** shows that the activity of catechin for scavenging $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ was inferior to that of quercetin, indicating the importance of the conjugated double bond system and the carbonyl group. The ORAC assay also suggested quercetin to be a more potent inhibitor of peroxyl radicals than catechin (Zhang et al. 2013). Yet, the positive impact of this structural feature relies mainly on the presence of both C_2-C_3 double bond and C_4-oxo group. Conflicting results were found

when comparing the anti-oxidative capacity of the compounds containing only the C_2-C_3 double bond or the C_4-oxo group. For example, taxifolin with C_4-carbonyl group but saturated C_2-C_3 bond exhibited lower TE (indicative of lower antioxidant capacity) and IC_{50} values (stronger antioxidant capacity) against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals than catechin. The similar findings were observed in comparison between naringenin and apigenin, as between taxifolin and quercetin (**Table 2**).

Compared to the critical structure features described above, hydroxyl groups in ring A are less significant. However, the hydroxyl groups in ring A increase the total number of hydroxyl groups in the molecule of flavonoids. Modification of these hydroxyl groups by methylation or glycosylation can suppress the anti-oxidative potency of flavonoids (**Table 2**). C_5-OH group conjugated with C_3-OH and C_4-oxo groups provide an importance site of trapping metal cations, such as iron and copper (Ferrali et al. 1997; Pietta, 2000).

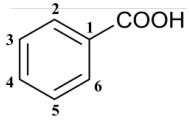
2.3.3 Tannins

As polymeric flavan-3-ols, proanthocyanidins follow the structure-activity relationship of flavonoids. Free C_3-OH group of C-ring and 3',4'-catechol group of B ring contribute to high antioxidant potential, and C_4-C_8 linkage ensures the stability of proanthocyanidins radicals formed in the scavenging process (Castillo et al. 2000). In addition to the structural features of monomeric units, the degree of polymerization (DP) influences the anti-oxidative activity of proanthocyanidins, likely due to the abundance of these three critical structural features presented above. Procyanidin trimers present more capacity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals than dimers and monomeric flavan-3-ols (**Table 2**). Vennat and co-workers extracted and fractionated procyanidins from tormentil (*Potentilla tormentilla*). The anti-oxidative activity of procyanidins against superoxide anions increased with the increasing DP in the order of dimers and trimers < tetramers < pentamers and hexamers (Vennat, Bos, Pourrat, Bastide, 1994). For peroxynitrite radicals, the strongest inhibitory effect was observed in the extract rich in procyanidin tetramers among those containing mono-, oligo-, and polymeric procyanidins. However, there was no clear correlation between the degree of polymerization and radical-scavenging (Arteel, Sies, 1999).

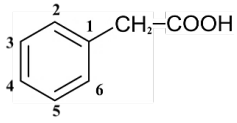
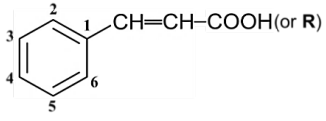
The anti-oxidative mechanism of ellagitannins included metal-chelating and free radical-scavenging. Chelation of metal cations may be the more important action compared to radical quenching, especially in the ion-induced lipid peroxidation (Gyamfi, Aniya, 2002). Moilanen *et al*. evaluated the anti-$OH^{\bullet}$ capacity of 13 ellagitannins in the deoxyribose assay, where $OH^{\bullet}$ radicals were produced by the presence of Fe^{2+} ions. The results suggested that the anti-

oxidative effect might be ascribed to the formation of Fe^{2+}-ellagitannin complexes instead of scavenging $OH^{\bullet}$ directly, since the contents of ellagitannins used in the measurement of chelation was 10-fold less than those used in the measurement of radical-scavenging activity (Moilanen, Karonen, Tähtinen, Jacquet, Quideau, Salminen, 2016). Hatano and Yokozawa proposed that the ability of ellagitannins for quenching $O_2^{\bullet-}$ and $DPPH^{\bullet}$ radicals was in proportion to the number of hydroxyl groups, and the presence of multiple free galloyl groups (Hatano et al., 1989; Yokozawa, Chen, Dong, Tanaka, Nonaka, Nishioka, 1998). The contribution of different structural features is dependent on the nature of radicals. For $DPPH^{\bullet}$ radicals, galloyl group was the main contributor, followed by HHDP, DHHDP, and chebuloyl groups, whereas the compounds with galloyl, and chebuloyl groups were potent for $OH^{\bullet}$ scavenging (Yoshida et al., 1989; Moilanen, Karonen, Tähtinen, Jacquet, Quideau, Salminen, 2016). In addition, the abundance of hydroxyl groups in ellagitannins can cause a strong pro-oxidative effect, even at a low concentration. This may explain some contradictory results from other antioxidant assays.

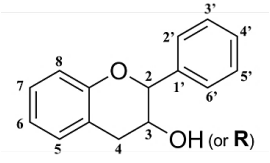
Table 2. Free radical scavenging capacity of some phenolic compounds measured ABTS and DPPH assays

Compounds	*Hydroxyl (OH) group substituents*		*Other substituents*	*Anti-oxidative capacity*	
	number of OH	*OH position*		*ABTS*	*DPPH*
Hydroxybenzoic acids 					
o-hydroxybenzoic acid (salicylic acid)	1	2-OH	-	0.04±0.01[a] 0.04±0.00[b]	0.05±0.00[b] 0±0[h]
m-hydroxybenzoic acid	1	3-OH	-	0.84±0.05[a] 0.03±0.00[b]	0.07±0.00[b] 0±0[h]
p-hydroxybenzoic acid	1	4-OH	-	0.08±0.01[a] 0.03±0.00[b] 0[c]	0.06±0.00[b] 0[d]
4-hydroxy-3-methoxybenzoic acid (vanillic acid)	1	4-OH	3-OCH_3	1.43±0.05[a] 0.09±0.00[b] 1.12[c] 2.1909[i]	0.06±0.00[b]
4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid)	1	4-OH	3,5-OCH_3	1.36±0.01[a] 1.39±0.02[b] 1.2[c] 4.1088[i]	1.33±0.01[b] 63[d] 12.3±0.0[e]
2,3-dihydroxybenzoic acid	2	2,3-OH	-	1.46±0.01[a]	46±3[h]
2,4-dihydroxybenzoic acid (*β*-resorcylic acid)	2	2,4-OH	-	1.22±0.02[b]	1.27±0.01[b] 0±0[h]
2,5-dihydroxybenzoic acid (gentisic acid)	2	2,5-OH	-	1.04±0.03 [a]	7.6±0.2[e] 31±0[h]
3,4-dihydroxybenzoic acid (protocatechuic acid)	2	3,4-OH	-	1.19±0.03[a] 1.15±0.01[b]	1.29±0.01[b] 11.1±0.0[e] 15.0[f] 2.2±0.1[g] 41±1[h]
3,5-dihydroxybenzoic acid (*α*-resorcylic acid)	2	3,5-OH	-	2.15±0.05[a]	1±0[h]

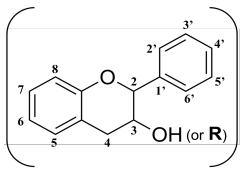
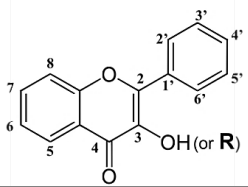
(**Table 2** continued)

Compounds	*Hydroxyl (OH) group substituents*		*Other substituents*	*Anti-oxidative capacity*	
	number of OH	*OH position*		*ABTS*	*DPPH*
3,4,5-trihydroxybenzoic acid (gallic acid)	3	3,4,5-OH	-	3.01±0.05[a] 3.52±0.03[b] 7.354[i]	3.92±0.03[b] 5.1±0.1[e] 12.0[f] 75±2[h]
Hydroxyphenylacetic acids 					
o-hydroxyphenylacetic acid	1	2-OH	-	0.99±0.09[a]	
m-hydroxyphenylacetic acid	1	3-OH	-	0.90±0.11[a]	
p-hydroxyphenylacetic acid	1	4-OH	-	0.34±0.10[a] 1.2017[i]	0±0[h]
4-hydroxy-3-methoxyphenylacetic acid	1	4-OH	3-OCH_3	1.72±0.06[a]	0.84±0.00[b]
2,5-dihydroxyphenylacetic acid	2	2,5-OH	-	0.91±0.05[a]	
3,4-dihydroxyphenylacetic acid	2	3,4-OH	-	2.19±0.08[a]	71±0[h]
Hydroxycinnamic acids and derivatives 					
2-hydroxycinnamic acid (*o*-coumaric acid)	1	2-OH	-	0.99±0.15[a] 0.93±0.01[b] 2.2009[i]	
3-hydroxycinnamic acid (*m*-coumaric acid)	1	3-OH	-	1.21±0.02[a] 0.82±0.00[b]	0.75±0.00[b]
3-hydroxy-4-methoxycinnamic acid (isoferulic acid)	1	3-OH	4-OCH_3	1.53±0.01[b]	1.24±0.01[b]
4-hydroxycinnamic acid (*p*-coumaric acid)	1	4-OH	-	2.22±0.06[a] 1.96±0.02[b] 1.5[c] 3.9137[i]	1.44±0.01[b] 0[d] 255±64[g]

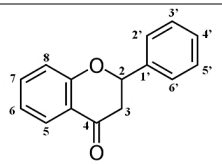
(**Table 2** continued)

Compounds	*Hydroxyl (OH) group substituents*		*Other substituents*	*Anti-oxidative capacity*	
	number of OH	*OH position*		*ABTS*	*DPPH*
4-hydroxy-3-methoxycinnamic acid (ferulic acid)	1	4-OH	3-OCH_3	1.90±0.02[a] 1.92±0.02[b] 1.66[c] 4.3967[i]	1.49±0.02[b] 40[d] 24.7±0.4[e] 4.9±0.1[g]
ferulic acid 4-*O*-sulfate	0	-	3-OCH_3, 4-OSO_3H	0.3805[i]	
ferulic acid 4-*O*-glucuronide	0	-	3-OCH_3, 4-*O*-glucuronide	0.0543[i]	
4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid)	1	4-OH	3,5-OCH_3	3.7791[i]	4.5±0.2[g]
2,4-Dihydroxycinnamic acid (umbellic acid)	2	2,4-OH	-		8.6±0.1[g]
3,4-dihydroxycinnamic acid (caffeic acid)	2	3,4-OH	-	1.26±0.01[a] 1.31±0.01[b] 2.4284[i]	1.24±0.01[b] 12.1±0.2[e] 2.6±0.1[g] 44±1[h]
5-*O*-caffeoylquinic acid (neochlorogenic acid)	2	4,5-OH	R=quinic acid group	1.56±0.01[b]	1.75±0.00[b]
3-*O*-caffeoylquinic acid (chlorogenic acid)	2	3,4-OH	R=quinic acid group	1.8891[i]	2.5±0.1[g] 93±4[h]
caffeic acid 3-*O*-glucuronide	1	4-OH	3-*O*-glucuronide	2.2477[i]	
caffeic acid 4-*O*-glucuronide	1	3-OH	4-*O*-glucuronide	0.7499[i]	
Flavan-3-ols 					
catechin	5	3, 5, 7, 3', 4'	-	2.40±0.05[a] 3.04±0.03[b]	2.95±0.04[b] 6.0±0.2[e] 18.19±0.93[j]
epicatechin	5	3, 5, 7, 3', 4'	-	2.50±0.02[a] 3.08±0.04[b]	3.18±0.02[b] 4.5±0.2[e] 16.09±0.41[j]

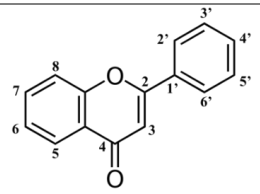
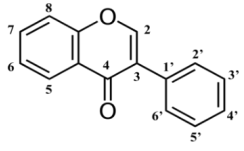
(**Table 2** continued)

Compounds	*Hydroxyl (OH) group substituents*		*Other substituents*	*Anti-oxidative capacity*	
	number of OH	*OH position*		*ABTS*	*DPPH*
epicatechin	5	3, 5, 7, 3', 4'	-	2.50±0.02[a] 3.08±0.04[b]	3.18±0.02[b] 4.5±0.2[e] 16.09±0.41[j]
epigallocatechin	6	3, 5, 7, 3', 4', 5'	-	3.80±0.06[a] 3.71±0.02[b]	3.56±0.02[b] 5.1±0.1[e]
catechin gallate	7	5, 7, 3', 4', three OH groups in R	R= galloyl group	5.25±0.02[b]	5.56±0.03[b]
epicatechin gallate	7	5, 7, 3', 4', three OH groups in R	R= galloyl group	4.90±0.02[a] 5.29±0.03[b]	5.26±0.02[b] 4.2±0.1[e]
epigallocatechin gallate	8	5, 7, 3', 4', 5', three OH groups in R	R= galloyl group	4.80±0.06[a] 5.95±0.17[b]	6.09±0.03[b] 3.6±0.0[e]
Proanthocyanidins  n					
procyanidin B-1 (dimer)	10 (n=2)	3, 5, 7, 3', 4'	-	6.14±0.12[b]	5.94±0.10[b] 3.2±0.0[e]
procyanidin B-2 (dimer)	10 (n=2)	3, 5, 7, 3', 4'	-		3.4±0.4[e]
procyanidin B-2 digallate (dimer)	14 (n=2)	5, 7, 3', 4', three OH groups in R	R= galloyl group	9.18±0.33[b]	8.79±0.24[b]
procyanidin C-1 (trimer)	15 (n=3)	3, 5, 7, 3', 4'		8.29±0.25[b]	7.93±0.35[b]
Flavonols 					

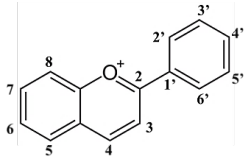
(**Table 2** continued)

Compounds	*Hydroxyl (OH) group substituents*		*Other substituents*	*Anti-oxidative capacity*	
	number of OH	*OH position*		*ABTS*	*DPPH*
kaempferol	4	3, 5, 7, 4'	-	1.34±0.08[a] 1.59±0.02[b]	1.32±0.01[b] 18.8±0.0[e] 28.05±0.28[j]
kaempferol 3-*O*-glucoside	3	5, 7, 4'	3-*O*-glucoside	0.14±0.02[b]	0.15±0.01[b]
quercetin	5	3, 5, 7, 3', 4'	-	4.70±0.1[a] 4.42±0.08[b]	4.60±0.02[b] 5.5±0.0[e] 10.89±0.03[j]
quercetin 3-*O*-rutinoside	4	5, 7, 3', 4'	3-*O*-rutinoside	2.40±0.06[a] 2.02±0.02[b]	2.33±0.03[b] 5.3±0.1[e] 9.40±0.31[j]
quercetin 3-*O*-glucoside	4	5, 7, 3', 4'	3-*O*-glucoside	2.39±0.02[b]	2.16±0.04[b] 9.45±0.06[j]
quercetin 3-*O*-galactoside	4	5, 7, 3', 4'	3-*O*-galactoside		10.01±0.00[j]
quercetin 3-*O*-rhamnoside	4	5, 7, 3', 4'	3-*O*-rhamnoside	2.18±0.02[b]	2.57±0.02[b]
quercetin 3-*O*-glucoside-7-*O*-rhamnoside	3	5, 3', 4'	3-*O*-glucoside, 7-*O*-rhamnoside	1.56±0.03[b]	1.63±0.03[b]
myricetin	6	3, 5, 7, 3', 4', 5'	-	3.10±0.30[a] 1.31±0.01[b]	1.38±0.01[b] 3.6±0.1[e]
Flavanone 					
naringenin	3	5, 7, 4'	-	1.53±0.05[a] 0.22±0.00[b]	0.14±0.00[b] >1000[j]
naringenin 7-*O*-rutinoside	2	5, 4'	7-*O*-rutinoside	0.76±0.05[a] 0.10±0.00[b]	0.08±0.00[b]
hesperetin		5, 7, 3'	4'-OCH_3	1.37±0.08[a] 0.40±0.02[b]	0.27±0.00[b] 236.63±0.86[j]
hesperetin 7-*O*-rutinoside	2	5, 3'	7-*O*-rutinoside, 4'-OCH_3	1.08±0.04[a] 0.10±0.00[b]	0.08±0.00[b] 281.41±2.62[j]
taxifolin	5	3, 5, 7, 3', 4'	-	1.90±0.03[a]	9.27±0.26[j]

(**Table 2** continued)

Compounds	*Hydroxyl (OH) group substituents*		*Other substituents*	*Anti-oxidative capacity*	
	number of OH	*OH position*		*ABTS*	*DPPH*
Flavones 					
chrysin	2	5, 7	-	1.43±0.07[a] 0.08±0.00[b]	0.05±0.00[b] 492.57±23.94[j]
apigenin	3	5, 7, 4'	-	1.45±0.08[a] 0.09±0.00[b]	0.04±0.00[b] 463.40±22.28[j]
apigenin 8-*C*-glucoside	3	5, 7, 4'	8-glucoside	0.22±0.00[b]	0.21±0.00[b]
apigenin 7-*O*-glucoside	2	5, 4'	7-*O*-glucoside	0.08±0.00[b]	0.05±0.00[b]
luteolin	4	5, 7, 3', 4'	-	2.10±0.05[a] 2.18±0.02[b]	2.24±0.02[b] 11.04±0.38[j]
luteolin 7-*O*-glucoside	3	5, 3', 4'	7-*O*-glucoside	1.47±0.00[b]	1.39±0.02[b] 28.17±0.69[j]
luteolin 4'-*O*-glucoside	3	5, 7, 3'	4'-*O*-glucoside	1.74±0.09[a]	
luteolin 3',7-*O*-diglucoside	2	5, 4'	3',7-*O*-diglucoside	0.79±0.04[a]	
Isoflavones 					
chrysin	2	5, 7	-	1.43±0.07[a] 0.08±0.00[b]	0.05±0.00[b] 492.57±23.94[j]
apigenin	3	5, 7, 4'	-	1.45±0.08[a] 0.09±0.00[b]	0.04±0.00[b] 463.40±22.28[j]
apigenin 8-*C*-glucoside	3	5, 7, 4'	8-glucoside	0.22±0.00[b]	0.21±0.00[b]
apigenin 7-*O*-glucoside	2	5, 4'	7-*O*-glucoside	0.08±0.00[b]	0.05±0.00[b]
luteolin	4	5, 7, 3', 4'	-	2.10±0.05[a] 2.18±0.02[b]	2.24±0.02[b] 11.04±0.38[j]

(**Table 2** continued)

Compounds	*Hydroxyl (OH) group substituents*		*Other substituents*	*Anti-oxidative capacity*	
	number of OH	*OH position*		*ABTS*	*DPPH*
luteolin 7-*O*-glucoside	3	5, 3', 4'	7-*O*-glucoside	1.47±0.00[b]	1.39±0.02[b] 28.17±0.69[j]
luteolin 4'-*O*-glucoside	3	5, 7, 3'	4'-*O*-glucoside	1.74±0.09[a]	
luteolin 3',7-*O*-diglucoside	2	5, 4'	3',7-*O*-diglucoside	0.79±0.04[a]	
genistein	3	5, 7, 4’	-	0.12±0.00[b]	0.10±0.00[b]
genistein 7-*O*-glucoside	2	5, 4'	7-*O*-glucoside	0.08±0.00[b]	0.03±0.00[b]
daidzein	2	7, 4’	-	0.10±0.00[b]	0.03±0.00[b]
daidzein 7-*O*-glucoside	1	4’	7-*O*-glucoside	0.07±0.00[b]	0.04±0.00[b]
glycitein	2	7, 4’	6-OCH_3	0.10±0.00[b]	0.02±0.00[b]
Anthocyanidins and anthocyanins 					
cyanidin	5	3, 5, 7, 3', 4'		4.40±0.12[a]	11.62±0.11[j]
cyanidin 3-*O*-rutinoside	4	5, 7, 3', 4'	3-*O*-rutinoside	3.25±0.1[a]	
cyanidin 3-*O*-galactoside	4	5, 7, 3', 4'	3-*O*-galactoside	2.90±0.03[a]	
delphinidin	6	3, 5, 7, 3', 4', 5'		4.44±0.11[a]	
pelargonidin	4	3, 5, 7, 4'	-	1.30±0.1[a]	
peonidin	4	3, 5, 7, 4'	3'-OCH_3	2.22±0.2[a]	
malvidin	4	3, 5, 7, 4'	3',5'-OCH_3	2.06±0.1[a]	
malvidin 3-*O*-glucoside	3	5, 7, 4'	3-*O*-glucoside, 3',5'-OCH_3	1.78±0.02[a]	

[a] The results were defined as the concentration of Trolox solution with equivalent antioxidant potential to a 1 mM concentration of the compound (mM); Rice-Evans, Miller, Paganga, 1996. [b] The results were defined as the concentration of Trolox solution with equivalent antioxidant potential to a 1 mM concentration of the compound (mM), The reaction for scavenging DPPH radicals was carried out for 120 min; Cai, Sun, Xing, Luo, Corke, 2006. [c] The results were expressed as mmoles of Trolox equivalents per mmole of phenolic acid (mM); Zhou, Yin, Yu, 2006. [d] The DPPH radical scavenging activities of phenolics were expressed as Inhibition of DPPH (%) for 1 min; Zhou, Yin, Yu, 2006. [e] The DPPH radical scavenging activities of phenolics were expressed as the concentration (10^{-6} M) of the compound to give a 50 % of DPPH• scavenging activities (EC_{50}); Garcia-Parrilla, Villano, Fernandez-Pachon, Moya, Troncoso, 2007. [f] The DPPH radical scavenging activities of phenolics were expressed as the concentration (10^{-6} M) of the compound to give a 50 % of DPPH• scavenging activities (EC_{50}); Reis, et al. 2010; [g] The DPPH radical scavenging activities of phenolics were expressed as the concentration (10^{-5} M) of the compound to give a 50 % of DPPH• scavenging activities (EC_{50}); Abramovic, Terpinc, 2010; [h] The DPPH radical scavenging activities of phenolics were expressed as Inhibition of DPPH (%) for 1 min; Sroka, Cisowski, 2003; [i] The results were expressed as the slope of dose-activity curve; Piazzon, Vrhovsek, Masuero, Mattivi, Mandoj, Nardini, 2012. [j] The DPPH radical scavenging activities of phenolics were expressed as the concentration (10^{-6} M) of the compound to give a 50 % inhibition of DPPH• (IC_{50}); Seyoum, Asres, El-Fiky, 2006.

2.4 Anti-bacterial activities of extracts of berry species

Table 3 presents some previous research on anti-bacterial activities of certain berry species. As reported previously by Rauha and co-workers, the fruit extracts of berry plants showed inhibitory effects on a wild range of foodborne pathogens, such as *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (Rauha, et al., 2000). The extracts of bilberry, cloudberry, crowberry, and raspberry inhibited the growth of all bacteria tested. Bog bilberry (*Vaccinium uliginosum*) was active only on gram-positive bacteria, and cowberry (i.e. lingonberry, *Vaccinium vitis-idaea*) was the least effective extract, showing slight inhibition against *B. subtilis* and *M. luteus* strains only. Among the tested bacteria, *M. luteus* strains were sensitive to most of the berry extracts tested but cranberry (*Vaccinium oxycoccos*). In contrast, *S. epidermidis* strains had higher resistance than other bacteria. The extracts of blackcurrant, chokeberry, cowberry, and small cranberry did not show any inhibitory effects on the growth of *S. epidermidis*.

Puupponen-Pimiä and co-workers studied the efficacies of eight common Finnish berries against *Bifidobacterium lactis*, *Enterococcus faecalis*, *E. coli* spp., *Lactobacillus* spp., and *Salmonella enterica* sv. Typhimurium (Puupponen-Pimiä, et al., 2001). The aqueous acetone (70 %) extracts of cloudberry, raspberry and strawberry had the strongest inhibitory efficacy on Gram-negative bacteria, especially on *S. enterica* sv. Typhimurium. Since the raw extracts contain a wild range of chemical compounds, it is difficult to pinpoint the main components contributing to anti-bacterial activity. After comparing the results with the anti-bacterial activity of reference compounds of phenolics, the authors suggested that phenolic compounds contributed to the anti-bacterial activity of these berry extracts. The major phenolic compounds were determined and fractionated in the latter study in order to determine the role of phenolics (Puupponen-Pimiä, et al., 2005). For *S. aureus*, phenolic compounds, especially ellagitannins, were the main contributors to the anti-bacterial activity of berry extracts. Nevertheless, most of the inhibitory effects against *Listeria monocytogenes* and *S. enterica* sv. Typhimurium strains were caused by the presence of other compounds, such as organic acids, mostly due to the decrease in pH. The impact of pH on growth of bacteria was also observed in the study of cranberry juice, where concentrated cranberry juice caused significant reduction in log CFU/mL of *E. coli* (O157:H7), *L. monocytogenes*, *S. enterica* sv. Typhimurium, and *S. aureus* (Wu, Qiu, Bushway, Harper, 2008). Both low pH value and phenolic compounds were responsible for cell deformation, breakage of cell wall and membrane, and condensation of cellular materials of the bacterial strains.

Côté and co-workers compared the activity of juice and fruits of cranberry against *E. coli*, *L. monocytogenes*, *P. aeruginosa*, *S. enterica* sv. Typhimurium, and *S. aureus* (Côté, Caillet, Doyon, Dussault, Sylvain, Lacroix, 2011). In this study, three phenolic extracts were obtained from frozen cranberry fruits, containing water-soluble phenolics, apolar phenolics (such as flavonols, flavan-3-ols, and proanthocyanidins), and anthocyanins, respectively. To remove the effect of pH, all berry extracts and juice sample were adjusted to neutral. Surprisingly, neutralized cranberry juice showed no suppressive effect on any pathogens studied. In the following test on growth inhibition kinetics, only *L. monocytogenes* and *S. aureus* strains were deactivated by exposure in the neutral juice for 30 min. This indicated that pH was the main contributor to capacity of cranberry juice against *E. coli* (O157:H7), *P. aeruginosa*, and *S. enterica* sv. Typhimurium. For the neutralized berry extracts, the highest inhibitory efficacy was found in the extract rich in water-soluble phenolics, expressed as low values of minimum inhibitory concentration (MIC) and maximal tolerated concentration (MTC). The strains of *P. aeruginosa* and *S. aureus* were sensitive to water-soluble phenolics, whereas *E. coli* (O157:H7), *L. monocytogenes*, and *S. enterica* sv. Typhimurium had strong tolerances.

Lacombe *et al*. fractionated the compounds in cranberry as sugars and organic acids, total phenolics, and anthocyanins before anti-*E. coli* (O157:H7) test (Lacombe, Wu, Tyler, Edwards, 2010). The results suggested that all fraction (diluted as a ratio of 1:2 and 1:4) exhibited a major growth inhibition against *E. coli*; however, after neutralization at pH 7.0, the fraction of sugars and organic acids had no anti-bacterial effect, whereas the total phenolics and anthocyanins retained generally antimicrobial effects in a neutral environment. The study also determined the mechanism of compounds inhibiting *E. coli* strains. As suggested by transmission electron microscopy (TEM), sugars and organic acids led to an increased osmotic stress because anthocyanins disintegrated the outer membrane. In another study of Lacombe *et al*., the raw extract of a lowbush blueberry (*Vaccinium angustifolium*) had a significant suppressive effect on the growth of *E. coli* (O157:H7), *L. monocytogenes*, *S. enterica* sv. Typhimurium, and *Yersinia enterocolitica* (Lacombe, Wu, White, Tadepalli, Andre, 2012). In order to compare the anti-bacteria activity of phenolics, blueberry extract were fractionated using C 18 and LH-20 Sephadex columns. The results suggested that the fractions rich in monomeric phenolics were the most effective against all bacteria studied; whereas the proanthocyanidin fraction showed only a limited growth inhibition on *L. monocytogenes* and *Y. enterocolitica*. *L. monocytogenes* strains were more sensitive to all blueberry fractions than *E. coli* and *S. enterica* sv. Typhimurium, based on the value of MIC and minimum bactericidal concentration (MBC). *S. enterica* sv. Typhimurium strains showed more resistance to proanthocyanidins

than monomeric phenolics and anthocyanins. High tolerance of *S. enterica* sv. Typhimurium strains to proanthocyanidins was also found in the study of lingonberry (*Vaccinium vitis-idaea*) and cranberry (*Vaccinium macrocarpon*). Isolated procyanidins strongly inhibited *S. aureus*, but no effect was shown on *S. enterica* sv. Typhimurium, *L. rhamnosus*, and *E. coli* strains (Kylli et al., 2011).

As by-products of berry processing, leaf and pomace have been considered for application in food industry as sources of natural preservatives. Silva and co-workers found that leaf extract of blueberry had better activity than fruit extracts regarding inhibition against methicillin resistant and sensitive *S. aureus* (Silva, et al., 2015). Chlorogenic acid was the major phenolic compound in both leaf and berry extracts, and might be responsible primarily for anti-bacterial effects. Leaf extracts altered the virulence property of *S. aureus* strains by deactivating DNase and coagulase. Salaheen *et al*. studied the impact of blueberry (*Vaccinium corymbosum*) and blackberry (*Rubus fruticosus*) pomace on *Campylobacter jejuni* strains (Salaheen, Nguyen, Hewes, Biswas, 2014). Phenolic compounds in blueberry pomace inhibited the growth of *C. jejuni* as well as interfered the gene expression of the strains and interaction with host cell. Moreover, the ethanolic extracts of the pomace influenced the cell surface hydrophobicity and auto-aggregation of *C. jejuni* strains, as well as their motility (swimming and swarming). These all resulted in the alteration of virulence of the strains. The juice-pressing residues of blackberry, blackcurrant, blueberry, and raspberry also presented significant growth inhibition against various pathogens (Widsten, Cruz, Fletcher, Pajak, McGhie, 2014).

The inhibitory efficacy against bacteria varies among the genotypes of berry plants. The aqueous ethanol extracts of blueberry of four cultivars were studied against *L. monocytogenes* and *S. enterica* sv. Enteritidis (Shen, et al., 2014). After incubation of 24 hours, the extracts (ethanol removed) of the cultivars ‘Elliott’ and ‘Darrow’ resulted in a remarkable growth reduction in *L. monocytogenes* at the concentration of 450 mg/mL, which was two-fold lower than the extracts of the cultivars ‘Bluecrop’ and ‘Duke’. *S. enterica* sv. Enteritidis population was reduced to levels under detection limits when the concentration of the extracts of ‘Elliott’ and ‘Darrow’ was increased to 900 mg/mL. In contrast, the cultivars ‘Bluecrop’ and ‘Duke’ showed no effect on *S. enterica* sv. Enteritidis strains. Compared to *S. enterica* sv. *Enteritidis*, *L. monocytogenes* strains were more susceptible to blueberry extract, as suggested also by the low MIC and MBC values. Since the impact of pH was excluded in this study, potent anti-bacterial activity of blueberry extracts might have been due to the abundance of phenolic compounds. The active antimicrobial compounds in the blueberry extracts might be chlorogenic acid, quercetin, ellagic acid, and quercetin 3-*O*-galactoside.

The cell structure of bacteria should also be considered in the research on the activities of berry extracts against pathogens. According to the report of Puupponen-Pimiä *et al*., all the studied phenolic extracts of Finnish berries showed strong inhibition against the growth of *E. coli* and *S. enterica* sv. Typhimurium, whereas the anti-*Lactobacillus* activities were only found in the extracts of raspberry, cloudberry, and blueberry at high content (Puupponen-Pimiä, et al., 2001). Wu *et al*. proposed that Gram-negative bacteria (*E. coli* O157:H7 and *S. enterica* sv. Typhimurium) were more sensitive to cranberry concentrate than Gram-positive pathogens, *L. monocytogenes and S. aureus* (Wu, Qiu, Bushway, Harper, 2008). The same species of bacteria were also studied by Côté *et al*. using berry extracts of cranberry (*Vaccinium macrocarpon*). The results indicated no correlation between Gram-positive or -negative strains and their sensitivity to phenolics in cranberry (Côté, Caillet, Doyon, Dussault, Sylvain, Lacroix, 2011). This may be related to the presence of the outer membrane in Gram-negative microbes (Gao, van Belkum, Stiles, 1999; Shan, Cai, Brooks, Corke, 2007). Performing as a permeability barrier, this hydrophilic surface is attributed to the lipopolysaccharide located in the outer leaflet of the membrane. The outer membrane contributed to the high tolerance of Gram-negative pathogens against hydrophobic antibiotics (Helander et al., 1998; Nikaido, 2003). Certain phenolic compounds in berry extracts were capable of disintegrating the outer membrane either by releasing lipopolysaccharide or by chelating divalent cations (Helander et al., 1998; Burt, 2004; Nohynek et al., 2006).

In addition, a few studies have reported previously that certain berry extracts exhibited neutral or positive effects on growth of probiotics (Gyawali, Ibrahim, 2012; Lacombe, Wu, White, Tadepalli, Andre, 2012; Yang, Hewes, Salaheen, Federman, Biswas, 2014). For instance, Lacombe and co-workers found that *Lactobacillus rhamnosus* strains were insensitive to phenolic fractions of blueberry with a low reduction in growth after inoculation. The following MIC and MBC tests also confirmed their high tolerance to the same fractions (Lacombe, Wu, White, Tadepalli, Andre, 2012). Yang *et al*. observed a growth increase of *L. rhamnosus* strains introduced by blackberry juice in two different broths (Yang, Hewes, Salaheen, Federman, Biswas, 2014).

Table 3. Previous studies on *in vitro* anti-bacterial activity of berry species

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
bayberry	*Myrica rubra*	fruit	total phenolics [a] total flavonoid [f] total tannins [g]	cylinder diffusion method	*Escherichia coli* *Listeria* spp. *Staphylococcus aureus* *Salmonella* spp. *Shigella* spp. *Streptococcus hemolyc* *Pseudomonas aeruginosa* *Vibrio parahaemolyticus*	Yao, Wang, Wang, Sun, Zhou, Luan, (2011)
bilberry	*Vaccinium myrtillus*	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Vaccinium myrtillus	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)
	Vaccinium myrtillus	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).

(**Table 3** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
blackberry	*Rubus fruticosus*	pomace	total phenolics [a]	microdilution broth method	*Salmonella* Typhimurium	Salaheen, et al., (2016)
	Rubus fruticosus	marc	total phenolics [a] total flavonoid [h] tannins [d]	gel diffusion assay	*Brochothrix thermosphacta* *Clostridia* spp. *Hafnia alvei* *Lactobacillus* spp. *Photobacterium phosphoreum* *Pseudomonas fluorescens* *Rahnella aquatilis* *Serratia* spp. *Shewanella putrefaciens*	Widsten, Cruz, Fletcher, Pajak, McGhie, (2014)
	Rubus fruticosus	juice	-	agar culture coupled with spectrophotometer analysis agar diffusion assay	*Escherichia coli* *Listeria monocytogenes* *Salmonella* Typhimurium	Yang, Hewes, Salaheen, Federman, Biswas, (2014)
	Rubus fruticosus	pomace	total phenolics [a]	microdilution broth method	*Campylobacter jejuni*	Salaheen, Nguyen, Hewes, Biswas, (2014)
blackcurrant	*Ribes nigrum*	marc	total phenolics [a] total flavonoid [h] tannins [d]	gel diffusion assay	*Brochothrix thermosphacta* *Clostridia* spp. *Hafnia alvei* *Lactobacillus* spp. *Photobacterium phosphoreum* *Pseudomonas fluorescens* *Rahnella aquatilis* *Serratia* spp. *Shewanella putrefaciens*	Widsten, Cruz, Fletcher, Pajak, McGhie, (2014)
	Ribes nigrum	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)

(**Table 3** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
	Ribes nigrum	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)
	Ribes nigrum	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)
	Ribes nigrum	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).
blueberry	*Vaccinium angustifolium*	pomace	total phenolics [a]	microdilution broth method	*Salmonella* Typhimurium	Salaheen, et al., (2016)
	Vaccinium angustifolium	fruit leaf	phenolic acids [d] anthocyanidins [d] flavonols [d]	well diffusion assay	methicillin resistant and sensitive *Staphylococcus aureus*	Silva, et al., (2015)
	Vaccinium spp.	marc	total phenolics [a] total flavonoid [h] tannins [d]	gel diffusion assay	*Brochothrix thermosphacta* *Clostridia* spp. *Hafnia alvei* *Lactobacillus* spp. *Photobacterium phosphoreum* *Pseudomonas fluorescens* *Rahnella aquatilis* *Serratia* spp. *Shewanella putrefaciens*	Widsten, Cruz, Fletcher, Pajak, McGhie, (2014)
	Vaccinium angustifolium	pomace	total phenolics [a]	microdilution broth method	*Campylobacter jejuni*	Salaheen, Nguyen, Hewes, Biswas, (2014)

(**Table 3** continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
	Vaccinium angustifolium (4 cultivars)	fruit	total phenolics [a] certain individual phenolics [d]	modified agar dilution method using tryptic soy instead of agar	*Listeria monocytogenes* *Salmonella* Enteritidis	Shen, et al., (2014)
	Vaccinium angustifolium	juice	-	modified agar dilution method using tryptic soy instead of agar	*Cronobacter sakazakii* spp.	Joshi, Howell, D'Souza, (2014)
	Vaccinium angustifolium	fruit	total phenolics [a] total anthocyanins [c] total proanthocyanidins [e]	agar diffusion assay	*Escherichia coli* *Lactobacillus rhamnosus* *Listeria monocytogenes* *Salmonella* Typhimurium *Yersinia enterocolitica*	Lacombe, Wu, White, Tadepalli, Andre, (2012)
	Vaccinium myrtillus	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)
cloudberry	*Rubus chamaemorus*	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Rubus chamaemorus	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)
	Rubus chamaemorus	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)

(Table 3 continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
	Rubus chamaemorus	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).
chokeberry	*Aronia melanocarpa*	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Aronia melanocarpa	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).
cranberry	*Vaccinium macrocarpon*	fruit juice	total phenolics [a]	96 well microtiter plate method and broth dilution assay	*Escherichia coli* *Listeria monocytogenes* *Salmonella* Typhimurium *Staphylococcus aureus* *Pseudomonas aeruginosa*	Côté, Caillet, Doyon, Dussault, Sylvain, Lacroix, (2011)

(Table 3 continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
	Vaccinium macrocarpon	fruit	proanthocyanidins [d]	liquid culture method	*Escherichia coli* *Lactobacillus rhamnosus* *Salmonella* Typhimurium *Staphylococcus aureus*	Kylli, et al., (2011)
	Vaccinium macrocarpon	fruit	total phenolics [a] sugars & organic acids [b] total anthocyanins [c]	96-well microplate method	*Escherichia coli*	Lacombe, Wu, Tyler, Edwards, (2010)
	Vaccinium macrocarpon	juice	total phenolics [a]	thin agar layer (TAL) plate method	*Escherichia coli* *Listeria monocytogenes* *Salmonella* Typhimurium *Staphylococcus aureus*	Wu, Qiu, Bushway, Harper, (2008)
	Vaccinium oxycoccus	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Vaccinium oxycoccus	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)
	n.g.	pomace	total phenolics [a] ellagic acid [d]	agar diffusion assay	*Escherichia coli* *Listeria monocytogenes* *Vibrio parahaemolyticus*	Vattem, Lin, Labbe, Shetty, (2004)
	Vaccinium oxycoccus	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)

(Table 3 continued)

Common name	***Latin name***	***Subject***	***Phenolic compounds***	***Test methods***	***Bacteria strains***	***Literature***
	Vaccinium oxycoccos	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).
crowberry	*Empetrum nigrum*	fruit	anthocyanins [d], flavonols [d], phenolic acids [d], flavan-3-ols [d], tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Empetrum nigrum	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).
grape	*Vitis rotundifolia*	pomace	total phenolics [a] anthocyanins [d], flavonols [d], phenolic acids [d], flavan-3-ols [d], tannins [d]	agar diffusion assay	*Escherichia coli* *Shigella sonnei* *Staphylococcus aureus* spp. *Salmonella* Typhimurium	Xu, Yagiz, Hsu, Simonne, Lu, Marshall, (2014).

(Table 3 continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
lingonberry	*Vaccinium vitis-idaea*	fruit	proanthocyanidins [d]	liquid culture method	*Escherichia coli* *Lactobacillus rhamnosus* *Salmonella* Typhimurium *Staphylococcus aureus*	Kylli, et al., (2011)
	Vaccinium vitis-idaea	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Vaccinium vitis-idaea	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)
	Vaccinium vitis-idaea	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)
	Vaccinium vitis-idaea	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).

(Table 3 continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
raspberry	*Rubus idaeus*	marc	total phenolics [a] total flavonoid [h] tannins [d]	gel diffusion assay	*Brochothrix thermosphacta* *Clostridia* spp. *Hafnia alvei* *Lactobacillus* spp. *Photobacterium phosphoreum* *Pseudomonas fluorescens* *Rahnella aquatilis* *Serratia* spp. *Shewanella putrefaciens*	Widsten, Cruz, Fletcher, Pajak, McGhie, (2014)
	Rubus idaeus	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Rubus idaeus	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)
	Rubus idaeus	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)

(Table 3 continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
	Rubus idaeus	fruit	-	hole-plate diffusion and cylinder diffusion methods	*Aspergillus niger* *Bacillus subtilis* *Candida albicans* *Escherichia coli* *Micrococcus luteus* *Pseudomonas aeruginosa* *Saccharomyces cerevisiae* *Staphylococcus aureus* *Staphylococcus epidermidis*	Rauha, et al., (2000).
rowanberry	*Sorbus aucuparia*	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
sea buckthorn	*Hippophaë rhamnoides*	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Hippophaë rhamnoides	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)

(Table 3 continued)

Common name	*Latin name*	*Subject*	*Phenolic compounds*	*Test methods*	*Bacteria strains*	*Literature*
	Hippophaë rhamnoides	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)
sloe berry	*Prunus spinosa*	puree	-	agar diffusion assay	*Salmonella* spp.	Gündüz, (2013)
strawberry	*Fragaria* × *ananassa*	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid culture coupled with fluorescence analysis	*Bacillus cereus* *Campylobacter jejuni* *Candida albicans* *Clostridium perfringens* *Escherichia coli* *Helicobacter pylori* *Lactobacillus* spp. *Salmonella enterica* spp. *Staphylococcus aureus* spp.	Nohynek, et al., (2006)
	Fragaria × *ananassa*	fruit	anthocyanins [d] flavonols [d] phenolic acids [d] flavan-3-ols [d] tannins [d]	liquid cultures by plate count method	*Lactobacillus rhamnosus* *Listeria* spp. *Salmonella enterica* spp. *Staphylococcus* spp.	Puupponen-Pimiä, et al., (2005)
	Fragaria × *ananassa*	fruit	total phenolics [a] anthocyanins [d] flavonols [d] OH-cinnamates [d] flavan-3-ols [d]	agar diffusion assay	*Bifidobacterium lactis* *Enterococcus faecalis* *Escherichia coli* spp. *Lactobacillus* spp. *Salmonella* Typhimurium	Puupponen-Pimiä, et al., (2001)

[a] total content of phenolics was measured by Folin−Ciocalteu assays; [b] sugars and organic acids were quantified as ratio of Brix and titratable organic acids; [c] total content of anthocyanins was measured by pH differential methods; [d] individual phenolic compounds were quantified by HPLC; [e] total content of proanthocyanidins was quantified by 4-dimethlylaminocinnamaldehyde (DMAC) method; [f] total content of flavonoids was determined by aluminium complex; [g] total content of tannins in the extracts was determined using a titrimetric method with potassium permanganate solution and indigo-carmine; [h] total content of flavonoids were determined by colorimetric assay.
"n.g." means "not given in the literature".

2.5 Mechanism of anti-bacterial activities of phenolics and structure-activity relationship

Generally, the mechanism of phenolic compounds against bacteria is (1) to react with cell membrane and increase the permeability causing breaking-down of cell; (2) to deactivate essential enzymes; or (3) a combination of both (1) and (2). Since phenolic compounds contain a remarkable deviation on structural arrangement, the antibacterial activity varies among different phenolic compounds as well as different pathogens (Puupponen-Pimiä, Nohynek, Alakomi, Oksman-Caldentey, 2005; Gyawali, Ibrahim, 2012).

Although enhanced by the low pH environment (Wen, Delaquis, Stanich, Toivonen, 2003), phenolic acids inhibit the growth of bacteria by diffusing across the membrane, which results in the acidified cytoplasm and thus the death of cells (Sánchez-Maldonado, 2014). The structural features should be taken into account when evaluating the anti-pathogenic capacity of phenolic acids, such as saturation on the alkyl side chain and ring substitution. Sánchez-Maldonado reported that hydroxycinnamic acids were generally more active than hydroxybenzoic acids with same number of hydroxyl group, regarding their impact on *Bacillus subtilis*, *E. coli, Lactobacillus plantarum*, and *Lactobacillus hammesii*. The unsaturation of the side chain lowers polarity of the molecule compared to corresponding hydroxybenzoic acids, and makes it easier for the compound to penetrate the cell membrane (Campos, Couto, Hogg, 2003). The MIC values of benzoic and hydroxybenzoic acids against *B. subtilis*, *E. coli, L. plantarum*, and *L. hammesii* strains were increased in the order: benzoic acid > *p*-hydroxybenzoic acid > protocatechuic acid > gallic acid. This suggested that the increasing number of hydroxyl groups on the phenol ring reduces the antibacterial activity of hydroxybenzoic acids (Sánchez-Maldonado, Schieber, Gänzle, 2011). The methylation at hydroxyl groups of phenol ring also affects the inhibition effect of hydroxybenzoic acids. For example, the MIC value of syringic acid (one hydroxyl and two methoxy groups) was lower than that of gallic acid but still higher than the activity of monohydroxybenzoic acids (Sánchez-Maldonado, Schieber, Gänzle, 2011). The negative impact of hydroxyl and methoxy groups was shown in hydroxycinnamic acids, but to a less extend compared to their benzoic counterparts (Sánchez-Maldonado, 2014). In addition, Wen *et al*. found that mixture of some selected phenolic acids had better activity against *L. monocytogenes* compared with any of the individual compounds of the mixture, suggesting synergy among different phenolic acids (Wen, Delaquis, Stanich, Toivonen, 2003). Certain acids, such as gallic acid, ferulic acid, and chlorogenic acid, were also found to exert a synergic effect with antibiotics (streptomycin) against Gram-negative pathogens (Saavedra, et al., 2010).

The anti-pathogenic activity of flavonoids may involve multiple mechanisms. Flavan-3-ols from green tea were found to interact with lipid bilayer and damage the cell membrane (Ikigai, Nakae, Hara, Shimamura, 1993). Flavonols such as galangin recognize the cytoplasmic membrane as a target site and induce the aggregation of bacterial cells (Cushnie, Hamilton, Chapman, Taylor, Lamb, 2007). Other evidence suggested that flavonoids could interfere with the activity of certain enzymes, exerting anti-bacterial activity (Bernard et al., 1997; Haraguchi, Tanimoto, Tamura, Mizutani, Kinoshita, 1998; Plaper, Golob, Hafner, Oblak, Solmajer, Jerala, 2003).

The anti-bacterial activity of flavonoids is associated with the number of hydroxyl group. Puupponen-Pimiä *et al*. suggested that the degree of hydroxylation of flavonoids might play an important role in suppressing the growth of both Gram-positive and Gram-negative bacterial species (Puupponen-Pimiä, et al., 2001). This was based on the observations that clear inhibitory effects against *Bifidobacterium lactis*, *Enterococcus faecalis*, and *Lactobacillus* spp. strains were not found in the aglycones of kaempferol and quercetin but in myricetin. Luteolin was an inhibitor against these bacteria, whereas no growth inhibition was shown in apigenin. Moreover, the capacity of flavonols and flavones against lactic acid bacteria was enhanced by increasing the number of hydroxyl groups at B ring (Puupponen-Pimiä, et al., 2001). For flavanones, as suggested by Tsuchiya, di-hydroxyl substitution at 2',4'- or 2',6'-position of B ring was essential for the activity against methicillin-resistant *Staphylococcus aureus*, as well as 5,7-dihydroxylation of A ring. The activity of hydroxyflavanones was also increased by the presence of certain aliphatic group at 6- or 8-position of A ring, which may facilitate the access to targeted microbe (Tsuchiya et al., 1996). Rauha *et al*. reported that flavanone was more potent reagent against *S. aureus* strains, compared with other flavonoid compounds (Rauha et al., 2000).

Compared with the aglycones, sugar moieties may diminish the activity of flavonols against bacteria (Rauha et al., 2000). Interaction among flavonoid compounds may have an impact on the inhibitory efficacy. As suggested by Mandalari, synergistic effect was found between eriodictyol and hesperetin in inhibiting the strains of *E. coli* and *S. enterica*, as well as between eriodictyol and naringenin against *S. enterica* and *Pseudomonas putida*. Hesperetin showed a minor antagonism with naringenin against *E. coli* and *S. enterica*, also with eriodictyol against *P. putida* (Mandalari et al., 2007).

Tannins in berry extracts exhibit anti-bacterial activity primarily by destabilizing cytoplasmic membrane, permeabilizing plasma membrane, inactivating the extracellular microbial enzymes, affecting directly on microbial metabolism or chelating the metal cations associated with microbial growth (Heinonen, 2007). To both gallotannins and ellagitannins, Gram-positive

pathogens are more sensitive than Gram-negative ones, which may be because the outer membrane of Gram-negative bacteria is more resistant to the permeabilizing and disintegrating impact of tannins (Puupponen-Pimiä et al, 2005; Nohynek et al., 2006; Engels, Schieber, Gänzle, 2011). However, this does not apply for the condensed tannins i.e. proanthocyanidins. This group of compounds was confirmed to restrain strongly the growth of *S. aureus*, but lacking efficacy against *S. enterica* sv. Typhimurium, *L. rhamnosus* and *E. coli* (Kylli et al., 2011). Little is known about the influence of structural features of the molecules on the anti-bacterial activity of tannins. Engels *et al*. proposed that the anti-bacterial activity of gallotannins depended on their affinity to iron, leading to the inactivation of membrane-bound proteins (Engels, Schieber, Gänzle, 2011). Therefore, structural arrangements, such as increasing the number of galloyl groups, that increase the iron-binding capacity of tannins may enhance the inhibition against foodborne pathogens. Some evidence suggest that polymerization of proanthocyanidins may be less important for the activities against certain bacteria. For example, Sivakumaran *et al*. fractionated proanthocyanidins from the leaves of forage legume based on the molecular weight. The selected rumen bacteria presented equal sensitivity to all proanthocyanidin fractions (Sivakumaran et al., 2004).

2.6 Summary and future prospects

As described previously in many studies, berry species are good sources of phenolic compounds. Since phenolics exert a wild range of health-promoting functions, fruits of berry plants have drawn great attention in scientific research and in commercial exploitation. Currently, some evidences have revealed that high level of phenolics is present in the by-products of berry plants, such as leaves, stems, and juice-pressing residuals, suggesting the potential of developing natural food preservatives and nutritional supplements from these materials.

For berry species, anti-oxidative and anti-bacterial activities have been investigated *in vitro* for decades. Antimicrobial activities of extracts of fruits, leaves, seeds and stems have been evaluated by multiple assays on a variety of bacteria, showing significant activities due to the abundance of phenolic compounds. Questions have been raised frequently on whether *in vitro* results of phenolics may correspond to *in vivo* activities. Compared to *in vivo* assays, *in vitro* studies are often simplified research models, which do not involve complicated interactions of many physiological processes (Rice-Evans, 2001). Both structure and content of phenolics reaching the targeted tissue may not be same as those in *in vitro* assays, which is among many factors causing the deviation between *in vitro* and *in vivo* measurements. Still, *in vitro* studies are

needed and often applied as fast and simple methods for screening potential functional compounds.

Nevertheless, regarding to the *in vitro* activity of berry plants, most of previous research has not been able to determine the main contributors of the extracts studied, mainly due to lack of systematic profiling of phenolic compounds. The previous studies have focused on either the total content of phenolics (or certain group of phenolics) measured by UV/Vis spectrophotometry or some individual compounds using HPLC-MS resolution. This limited compositional information makes it impossible to pinpoint the major inhibitors against oxidants and foodborne pathogens. It is necessary to obtain a thorough phenolic profile from fruits and leaves of berry plants in order to optimize the process for obtaining extracts of high biological activities.

On the other hand, for berry plant extracts, the determination of main contributor of bioactivities is difficult. Some researchers have suggested to first isolate and purify the individual compounds from berry extracts, and then to compare the activity of each compound. Considering most of the extracts as a complex mixture of a large number of phenolics, the work would be time-consuming; also, this approach may neglect the synergic and antagonistic effects among different compounds. Based on this, multivariate statistical models may be a good solution, such as principal components analysis (PCA), and partial least squares regression (PLS). These two methods have shown advantages in the studies of sensory evaluation and climatic effects on phenolic composition (Zheng, Yang, Tuomasjukka, Ou, Kallio, 2009; Laaksonen, Mäkilä, Tahvonen, Kallio, Yang, 2013). Although without pinpointing directly the primary compounds, the multivariate models could reveal the distribution of phenolic compounds in different samples, and locate the major contributors based on their correlation to bioactivities. Moreover, these models indicate the potential interaction among chemical compounds regarding specific bioactivities. Combined with chromatographic methods, this approach may assist the development of new natural food preservatives from materials based on berry plants.

3 AIMS OF THE STUDY

The general aim of this research was to evaluate the possibility of using phenolic extracts from common Finnish berry plants as natural preservatives for food products (**Figure 1**). Phenolic compounds were extracted from berries and leaves of selected species/cultivars of berry plants with food grade aqueous ethanol. The phenolic profiles of the extracts were investigated systematically with HPLC-DAD and HPLC-MS. The *in vitro* activities of the extracts against free radicals and bacteria were analyzed by different assays. Fractionation of selected phenolic extracts was performed using column chromatography and the phenolic composition and bioactivities of the fractions were analyzed. Multivariate analysis models were established to pinpoint the main phenolic compounds responsible for the bioactivities. The influence of genotypes on the phenolic profiles of blackcurrant berries was studied.

The specific targets of the individual studies are the following:

1) To establish phenolic profile of aqueous ethanolic extracts of branches, fruits, and leaves of common berry species in Finland (**Study I**);

2) To evaluate *in vitro* anti-oxidative and antibacterial activities of the extracts, and to compare the contribution of the main groups of phenolics to the bioactivities studied (**Study II**);

3) To fractionate the phenolic extracts of selected berry plants, and to determine the correlation between individual phenolic compounds and bioactivities studied (**Study III**);

4) To estimate the variation in phenolic profiles among cultivars of blackcurrant fruits (**Study IV**).

4 MATERIALS AND METHODS

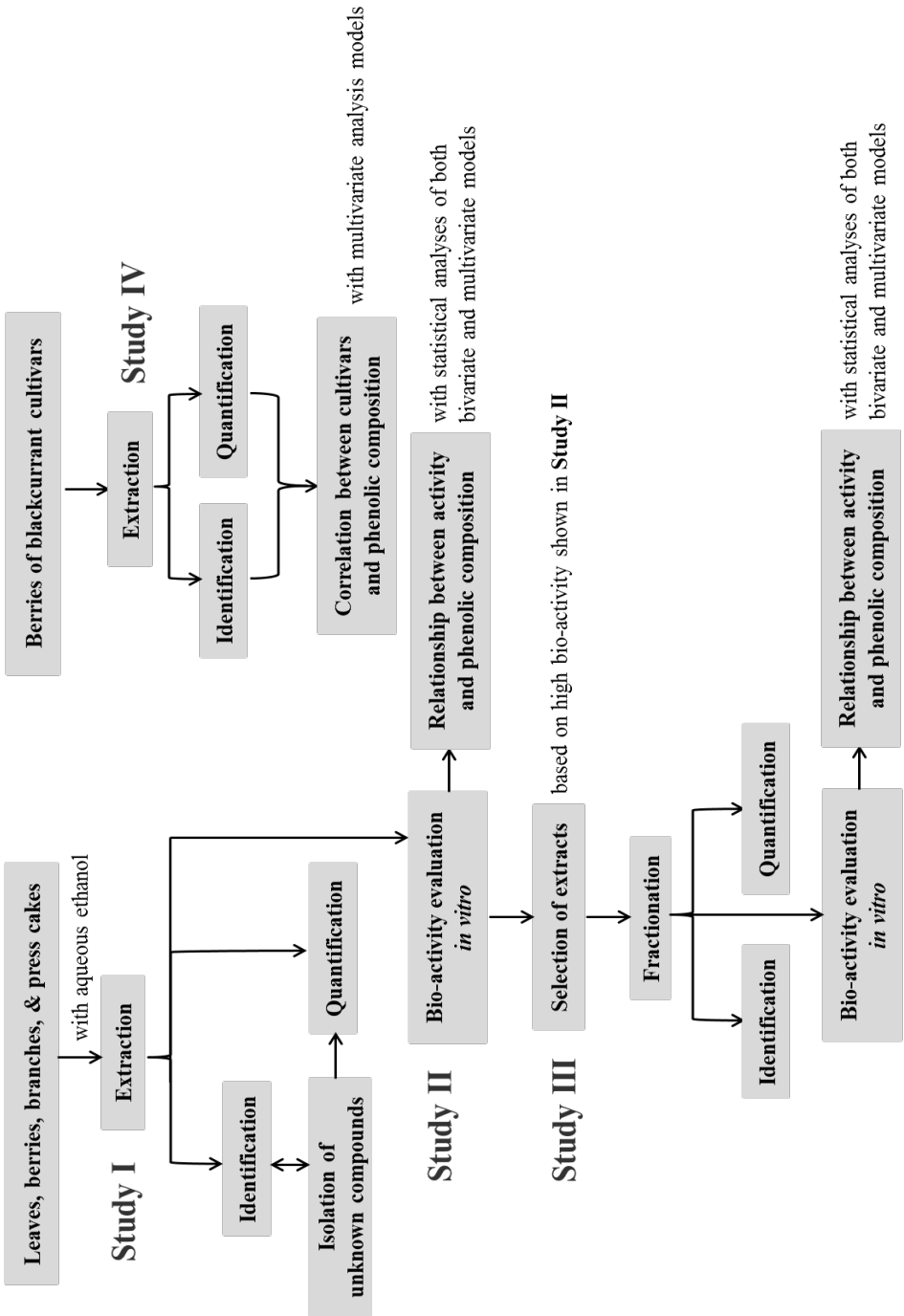


Figure 1. The scheme of analyses in the presenting research.

4.1 Plant materials

The samples used in Study **I**, **II**, and **III** were berries (12 kinds), leaves (12), branches (1), and press cakes (2) of thirteen Finnish berry plants. It included bilberry (*Vaccinium myrtillus*), chokeberry (*Aronia melonocarpa*), cranberry (*Vaccinium oxycoccos*), crowberry (*Empetrum nigrum*), currants (*Ribes nigrum* 'Mortti'& 'Vertti'; *Ribes rubrum* 'Red Dutch' & 'White Dutch'), hawthorn (*Crataegus grayana*), lingonberry (*Vaccinium vitis-idaea*), raspberry (*Rubus idaeus*), rowanberry (*Sorbus aucuparia*), saskatoon (*Amelanchier alnifolia*), and sea buckthorn (*Hippophaë rhamnoides* 'Terhi'&'Tytti').

Blackcurrant cultivars in Study **IV** were originated from Scotland ('Ben Dorain', 'Ben Gairn', 'Ben Hope', 'Ben Starav', 'Ben Tirran', 'Ben Tron', 'S 18/2/23', 'Ben Finlay', & '9154-3'), Lithuania ('Almiai', 'Dainiai', 'Gagatai', 'Joniniai', & 'Tauriai'), Latvia ('Mara'), Finland ('Marski', 'Mikael', 'Mortti', 'Vilma', & 'Venny') and Poland ('Tisel').

The detail information on plant materials has been given in **Table 4**. All berry samples were collected by professional horticulturists at optimally ripe, as defined by color, flavor, and structure. The pooled leaf (green) and branch samples were collected from multiple sites and different sides of the bushes or trees. The samples were all stored at -20 °C till analyzed.

Table 4. Information of plant materials studied. Reprinted from the original publication **I** (Tian, Liimatainen, Alanne, Lindstedt, Liu, Sinkkonen, Kallio, & Yang, 2017) with permission from Elsevier.

sample name	**Latin name**	**Collection site / source**
lingonberry and leaf bilberry and leaf	*Vaccinium vitis-idaea* *Vaccinium myrtillus*	Laarikallio Rauma, Paattinen, Turku, Finland
chokeberry and leaf rowan berry raspberry leaf redcurrant and leaf whitecurrant and leaf greencurrant and leaf	*Aronia melonocarpa* *Sorbus aucuparia* *Rubus idaeus* *Ribes rubrum* 'Red Dutch' *Ribes rubrum* 'White Dutch' *Ribes nigrum* 'Vertti'	Natural Resources Institute Finland, Piikkiö, Finland
blackcurrant leaf	*Ribes nigrum* 'Mortti'	
blackcurrant	*Ribes nigrum* 'Ben Dorain', 'Ben Gairn', 'Ben Hope', 'Ben Starav', 'Ben Tirran', 'Ben Tron', 'S 18/2/23', 'Ben Finlay', '9154-3', 'Almiai', 'Dainiai', 'Gagatai', 'Joniniai', 'Tauriai', 'Mara', 'Marski', 'Mikael', 'Mortti', 'Vilma', 'Venny' & 'Tisel'	
blackcurrant press cake	*Ribes nigrum* 'Mortti'	Saarioinen Oy, Finland
hawthorn and leaf	*Crataegus grayana*	Campus of University of Turku, Turku, Finland
sea buckthorn and leaf	*Hippophaë rhamnoides* 'Terhi' & 'Tytti'	Sammalmäki, Turku, Finland
saskatoon berry, leaf, and branch	*Amelanchier alnifolia*	Linnan Marjatila Oy, Lohja, Finland
crowberry cranberry press cake	*Empetrum nigrum* *Vaccinium oxycoccos*	Marjajaloste Meritalo Oy, Ylönkylä, Finland

4.2 Sample preparation

4.2.1 Extraction of phenolic compounds (Study I, III, and IV)

In the sub-studies **I** and **III**, the frozen samples were ground into powder with assistance of liquid nitrogen, and then extracted with acidic aqueous ethanol (ethanol:water:acetic acid, 70:30:1, v/v/v). The extraction was carried out with the aid of ultra-sonication and mechanical shaking at room temperature. The supernatant was collected after centrifugation (4420 × *g*). Two extraction methods were applied in Study **IV** according to the nature of phenolic compounds. Anthocyanins were extracted from berry slurry with acidified methanol (methanol: hydrochloric acid, 99:1, v/v) as reported in a previous study (Mäkilä et al. 2016). Other phenolic compounds were extracted with ethyl acetate. Both extractions were conducted four times, assisted with ultra-sonication and centrifugation.

4.2.2 Isolation of unknown compounds (Study I)

Several unknown compounds of high abundance from the extracts of Saskatoon leaves, Saskatoon berries, and raspberry leaves, were selected for further analyses. Both leaf and berry samples were extracted with ethyl acetate, following the same procedure as described in **4.2.1**. The supernatants were evaporated at 65°C, and the residue was dissolved into ethyl acetate. The unknown compounds were collected after separation with semi-preparative HPLC. The collected samples were later analyzed with NMR for structure determination.

4.2.3 Fractionation of phenolic compounds (Study III)

Eight acidified ethanol extracts were selected for fractionation based on high bioactivities observed in Study **II**, including leaf extracts of lingonberry, whitecurrant, hawthorn, saskatoon, and sea buckthorn (‘Tytti’) as well as berry extracts of sea buckthorn (‘Tytti’), chokeberry, and crowberry. Fractionation was performed using Sephadex LH-20 column chromatography. The elution was conducted successively with Milli-Q water, aqueous ethanol (including 20, 40, 70 and 90% ethanol, respectively) and aqueous acetone (50 and 90% acetone, respectively) at room temperature. Altogether, 22 fractions acquired from each extract were lyophilized and weighed. Phenolic compounds of these fractions were identified with HPLC-DAD and HPLC-MS, and quantified with HPLC-DAD.

4.3 Identification and quantification of phenolic compounds

4.3.1 Liquid chromatography analysis (Study I, III, and IV)

Three liquid chromatography (LC) systems were applied in the study **I**, **III**, and **IV**. The unknown compounds in Study **I** was isolated using an semi-preparative high-performance liquid chromatography (HPLC) system (Shimadzu Corp., Kyoto, Japan) equipped with a Phenomenex Aeris peptide XB-C18 column (250 × 10 mm, 5 µm, Torrance, CA, U.S.A.). A total flow rate of 3 mL/min and an injection volume of 100 µL were applied. Identification of phenolics was performed on an ultra-performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, U.S.A.). Quantification of phenolic compounds was carried out with a Shimadzu LC-10AT liquid chromatograph system (Shimadzu Corp., Kyoto, Japan). A Phenomenex Aeris peptide XB-C18 column (150 × 4.60 mm, 3.6 µm, Torrance, CA) was applied in both qualitative and quantitative analyses. The total flow rate was set at 1.0 mL/min and the sample injection volume was 10 µL. Anthocyanins and other phenolic compounds were analyzed separately. The mobile phase for anthocyanin analysis was a combination of water (A) and acetonitrile (B), both containing 5.0% (v/v) of formic acid; whereas analysis of other phenolics were performed using water (A) and acetonitrile (B) acidified with 0.1% of formic acid. LC gradient program varied in different sub-studies in order to acquire sufficient separations. The chromatograms were recorded at different wavelengths (280 nm for all phenolic compounds, 320 nm for hydroxycinnamic acids, 360 nm for glycosylated flavonols and flavones, and 520 nm for anthocyanins).

The compounds were quantified by the calibration curves of external standards. The calibration curves were established between peak area of standards in the HPLC chromatography and their concentration. For the compounds without corresponding reference standards, the calibration curves were chosen from the compounds with closest structures.

4.3.2 Mass spectrometric analysis (Study I, III, and IV)

Mass spectrometer (Waters Quattro Premier) was equipped with Waters Acquity UPLC system, a 2996 DAD detector and an electrospray ionization interface (Waters Corp., Milford, MA, U.S.A.). For the ESI-MS system in all sub-studies, the source temperature was set to 120 °C, and desolvation temperature was 300 °C. Both negative and positive ion modes were applied in the MS analysis for identification. Capillary voltage, cone voltage, and extractor voltage in negative ion mode were 3.5 kV, 35 V, and 7 V,

respectively, and 4.0 kV, 22 V, and 3 V in positive ion mode. The ions were monitored ranging from 100 to 1000 *m/z*. Tandem MS was used to determine the precursor and product ions of the analytes. The collision energy and cone voltage for MS^2 were 30 V and 22 V, respectively. The MS data processing was performed with Masslynx 4.1 software (Waters Corp., Milford, MA, U.S.A.).

4.3.3 Nuclear magnetic resonance analysis (Study I and III)

^1H NMR analyses were carried out on a Bruker Avance 500 spectrometer (Study **I**) and 600 spectrometers (Study **III**) operating at 500.13 and 600.13 MHz, respectively. Both spectrometers were equipped with a broadband inverse auto-tune probe (BBI-5 mm-Zgrad-ATM). The program was set with 256 scans, an acquisition time of 3.28 s, a spectral width of 10 kHz and 64 k data points. Other one- and two-dimensional NMR experiments, such as ^{13}C, 1D TOCSY, DQF-COSY, HSQC and HMBC, were performed for selected plant extracts and fractions. NMR spectra were processed with TopSpin 3.2 software (Bruker Corp., Billerica, MA, U.S.A.). The chemical shifts were referenced to the internal standard TSP at 0.00 ppm in Study **I**, whereas to an acetone resonance at 2.05 ppm in Study **III**.

4.4 *In vitro* assays of anti-oxidative activities (Study II and III)

The extracts and fractions were tested *in vitro* with four different anti-oxidative assays. Folin-Ciocalteu measurement was applied for general estimation of total phenolics in samples, and the results were calculated as the equivalents of gallic acid. DPPH assay was performed according to the method of Xie and Schaich (Xie, & Schaich, 2014). As suggested by Apak (Apak et al., 2013), the measurement time of DPPH assay was set at 0.5 min, 1 min, 2 min, and 10 min to simulate $OH^{\bullet}$, $OOH^{\bullet}$, and $NO^{\bullet}$ radicals. For ORAC assay, the procedure used was as described previously by Prior and Ou (Prior et al., 2003; Ou, Hampsch-Woodill, & Prior, 2001). ORAC assay was conducted to measure the capacity of the samples for quenching peroxyl-radicals, as well as total radical trapping antioxidant parameter (TRAP) assay. Both of these methods were designed using AAPH (2, 2'-azobis(2-amidinopropane) dihydrochloride) as hydrophilic initiator; however, the detective probe was fluorescein solution in ORAC but luminal solution in TRAP. In both assays, the results were expressed as Trolox equivalents.

4.5 *In vitro* study of anti-bacterial activities (Study II and III)

The anti-bacterial activities of the samples were evaluated on Gram-positive and -negative foodborne pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Salmonella enterica* sv. Typhimurium. All microbes were provided by VTT Culture Collection. The evaluation was carried out using the method described previously (Alakomi et al., 2007). In order to exclude the effects of acidic ethanol, each sample was re-suspended into the same volume of sterile Milli-Q water after evaporating the solvent. The bacterial cells grown at 37 °C overnight were diluted and inoculated with 10^5 cells per well, and the samples were tested at two different concentrations (10 and 20 µL per well). After incubation for 48 hours at 37 °C, the growth inhibition (%) of the samples against target microbes was calculated based on comparison with the growth of the control wells without additions of extracts.

4.6 Statistical analyses

Data in this research was expressed as mean ± standard deviation (SD). Correlation analyses were applied to investigate the contribution of phenolic compounds to bioactivities (Study **II** and **III**) and the variation of phenolics among berries of different blackcurrant cultivars (Study **IV**). Statistical analysis was conducted by IBM SPSS Statistics 24 (SPSS Inc., NY, U.S.A.) and Unscrambler 10.4 (Camo Process AS, Oslo, Norway).

5 RESULTS AND DISCUSSION

5.1 Phenolic profiles in Finnish berry species

5.1.1 Phenolic composition in extracts of Finnish berry species (Study I)

All together 160 compounds were identified or preliminarily identified from branches, fruits, and leaves of thirteen species, including flavan-3-ols (2 compounds), proanthocyanidins (4), ellagitannins (8), phenolic acids (22), flavonols (85), flavones (4), flavanones (2) and anthocyanins (27). Phenolic acids were characterized mainly as hydroxycinnamic acid conjugates such as caffeic acid, coumaric acid, and ferulic acid. Flavonols consisted of the derivatives of quercetin, isorhamnetin, kaempferol, myricetin, syringetin, and laricitrin. The main anthocyanidins were cyanidin, delphinidin, petunidin, peonidin, and malvidin. The identification of individual compounds was published in paper **I** (Tian et al., 2017).

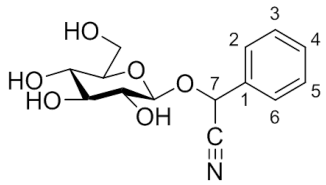
The leaf extracts had higher total concentrations of phenolics than berry and branch extracts (**Table 5**). Yet, the dominating phenolic compounds differed among the extracts studied. As the dominant phenolics in dark-skinned berries, anthocyanins represented for 95 % of the sum content of phenolics in bilberry, 89 % in blackcurrant press cake, and 81 % in crowberry. Ellagitannins represented 90 % of the sum content of phenolics in the extracts of sea buckthorn leaves and 70 % in raspberry leaves, respectively. *β*-*p*-Arbutin was the major compound in the leaf extract of lingonberry (271 mg/100 mL). The extract also contained high content of proanthocyanidins identified as procyanidin dimers and trimers (85 mg/100 mL) and flavan-3-ols (118 mg/100 mL) as (+)-catechin and (-)-epicatechin. The bilberry leaf extract was rich in hydroxycinnamic acid derivatives, primarily as 3-*O*-caffeoylquinic acid (82 % of sum content of phenolics). The derivatives of phenolic acids were present in some berry extracts at high levels, such as in Saskatoon berry, chokeberry, rowanberry, and lingonberry. High contents of flavonols were quantified in lingonberry leaves, raspberry leaves, and saskatoon leaves.

Some aromatic compounds were characterized based on NMR spectra (**Figure 2**). Prunasin was found at high levels in the chokeberry leaf extract, as well as the extract of saskatoon leaves and branches. Both white and red currant leaf extracts contained high amount of tyramine. Therefore, these extracts should be estimated from safety point view when considered as potential raw materials of food and food additives.

Table 5. Concentrations of phenolic compounds (mg/100 mL) in the fruit, leaf, and branch extracts measured by HPLC-DAD. Reprinted from the original publication **II** (Tian, Puganen, Alakomi, Uusitupa, Saarela, & Yang, 2018) with permission from Elsevier.

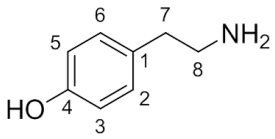
Extract name	*Flavan-3-ols*	*Proanthocyanidins*	*Ellagitannins*	*Phenolic acids*	*Flavonol glycosides*	*Flavone glycosides*	*Flavanone glycosides*	*Anthocyanins*	*Others*	*Sum*
Berry extracts										
lingonberry	11.3±1.2	4.0±0.3	-	21.2±1.1	3.2±0.1	-	-	4.8±0.1	-	44.5±2.8
bilberry	-	-	-	-	3.0±0.0	-	-	53.5±1.4	-	56.5±1.4
chokeberry	-	-	-	24.8±0.3	6.1±0.1	-	-	40.2±0.9	-	71.1±1.3
sea buckthorn 'Terhi'	-	-	-	-	8.6±0.1	-	-	-	-	8.6±0.1
sea buckthorn 'Tytti'	-	-	-	-	7.7±0.2	-	-	-	-	7.7±0.2
saskatoon	-	-	-	27.3±0.5	5.6±0.1	-	-	22.2±0.5	-	55.1±1.1
crowberry	-	-	-	2.7±0.1	5.7±0.2	-	-	34.9±0.5	-	43.3±0.8
rowanberry	0.3±0.0	0.3±0.0	-	23.7±0.6	3.4±0.1	-	-	0.5±0.0	-	28.2±0.7
Press cake extracts										
blackcurrant	-	-	-	1.0±0.0	3.4±0.0	-	-	33.7±0.5	-	38.1±0.5
cranberry	-	-	-	4.0±0.1	8.3±0.1	-	-	5.1±0.1	-	17.4±0.3
Leaf extracts										
lingonberry	117.8±3.2	85.1±2.3	-	39.9±1.5	99.9±2.3	-	-	-	271.1±2.0	613.8±11.3
bilberry	4.4±1.0	11.2±1.0	-	136.3±5.8	15.3±0.8	-	-	-	-	167.2±8.6
redcurrant	-	-	-	4.5±0.1	51.7±1.2	-	-	-	-	56.2±1.3
whitecurrant	2.4±0.1	-	-	6.5±0.6	36.4±0.8	1.9±0.3	-	-	7.8±0.1	55.0±1.9
greencurrant	2.9±0.0	-	-	2.4±0.0	49.0±0.5	-	-	-	-	54.3±0.5
hawthorn	19.3±1.9	23.5±1.6	-	14.0±1.0	47.1±0.4	16.1±0.4	-	-	-	120.0±5.3
chokeberry	-	-	-	18.2±0.2	31.0±0.3	-	-	-	7.7±0.1	56.9±0.6
sea buckthorn 'Terhi'	21.8±1.1	-	730.2±25.2	-	33.9±1.3	-	-	-	-	785.9±27.6
sea buckthorn 'Tytti'	25.6±0.7	-	548.9±19.5	-	31.1±1.4	-	-	-	-	605.6±21.6
raspberry	-	-	149.5±4.5	-	69.1±2.6	-	-	-	-	218.6±7.1
saskatoon	9.1±0.5	23.4±1.7	-	54.4±3.8	67.0±0.8	-	-	-	-	153.9±6.8
Branch extracts										
saskatoon	16.6±0.9	21.1±0.6	-	7.0±0.3	5.0±0.2	-	1.6±0.0	-	-	51.3±2.0

a



Prunasin		^{1}H (δ/ppm)	multiplicity (*J*/Hz)	^{13}C (δ/ppm)
aglycone	1	-	-	136.1
	2	7.58	m	130.6
	3	7.49	m	132.2
	4	7.49	m	133.2
	5	7.49	m	132.2
	6	7.58	m	130.6
	7	5.90	s	70.9
	8	-	-	121.5
glucose	1'	4.37	d (7.4)	104.1
	2'	3.28–3.36	m	72.6-79.8
	3'	3.28–3.36	m	72.6-79.8
	4'	3.28–3.36	m	72.6-79.8
	5'	3.28–3.36	m	72.6-79.8
	6'	3.73	dd (5.7, -12.2)	64.2
		3.91	dd (2.3, -12.2)	

b



Tyramine		^{1}H (δ/ppm)	multiplicity (*J*/Hz)	^{13}C (δ/ppm)
	1	-	-	130.9
	2	7.13	d (8.4)	132.9
	3	6.81	d (8.4)	118.6
	4	-	-	158.7
	5	6.81	d (8.4)	118.6
	6	7.13	d (8.4)	132.9
	7	2.88	t (8.0)	35.2
	8	3.14	t (8.0)	44.0

Figure 2. Structural information of aromatic compounds identified by NMR. Reprinted from the original publication **I** (Tian, Liimatainen, Alanne, Lindstedt, Liu, Sinkkonen, Kallio, & Yang, 2017) with permission from Elsevier.

5.1.2 Phenolic composition in fractions of Finnish berry species (study III)

Table 6 presented the main groups of phenolics in the fractions 1.2–4.2 of the extracts. The concentration of these groups was the sum content of individual compounds quantified by HPLC. In general, fractions 1.2–4.2 contained the derivatives of hydroxycinnamic acids. Anthocyanins and glycosylated flavonols were concentrated in the fractions 2.2–4.2. Flavones and flavanones were characterized mainly as apigenin and eriodictyol, respectively, as both glycosides and free aglycones. Low molecular weight proanthocyanidins (such as dimers and trimers) and ellagitannins were identified from the fractions 3.2–4.2, as well as the monomers of flavon-3-ols. Fractions 5–7 mostly consisted of proanthocyanidins or ellagitannins with higher molecular masses based on broad resonance humps of tannins in ^{1}H NMR and typical fragment ions observed in negative ion ESI-MS spectra (catechin fragment at *m/z* 289 and ellagic acid fragment at *m/z* 301). Nevertheless, these compounds could not be determined individually due to complex composition

Table 6. Concentrations of phenolic compounds (mg/100 mL) in the fractions of selected berry and leaf extracts.

Fraction No.	*Flavan-3-ols*	*Proanthocyanidins*	*Ellagitannins*	*Phenolic acids*	*Flavonol glycosides*	*Flavone glycosides*	*Flavanone glycosides*	*Anthocyanins*	*Others*	*Sum*
chokeberry										
fraction 1.2	-	-	-	14.8±0.2	-	-	-	-	-	14.8±0.2
fraction 2.1	-	-	-	40.4±0.2	-	-	-	-	-	40.4±0.2
fraction 2.2	-	-	-	36.9±0.1	-	-	17.7±0.2	0.3±0.0	-	54.9±0.4
fraction 3.1	-	-	-	8.8±0.2	1.9±0.0	-	13.9±0.0	16.6±0.1	-	41.2±0.2
fraction 3.2	-	-	-	10.7±0.2	11.6±0.1	-	-	150.9±0.2	-	173.2±0.5
fraction 4.1	-	-	-	1.9±0.0	8.3±0.1	-	-	27.3±0.1	-	37.6±0.2
fraction 4.2	-	-	-	13.7±0.1	-	-	2.1±0.1	2.0±0.1	-	17.8±0.3
crowberry										
fraction 1.2	-	-	-	34.6±0.3	-	-	-	-	-	34.6±0.3
fraction 2.1	-	-	-	18.8±0.1	-	-	-	3.1±0.1	-	22.0±0.2
fraction 2.2	-	-	-	-	0.6±0.0	16.5±0.3	-	192.9±1.2	-	210.0±1.5
fraction 3.1	-	-	-	-	2.0±0.0	16.5±0.2	-	476.6±0.9	-	495.1±1.1
fraction 3.2	-	-	-	-	4.1±0.2	0.4±0.1	-	60.2±1.2	-	64.7±1.4
fraction 4.1	14.3±0.1	-	-	10.8±0.1	23.5±0.2	-	-	12.4±0.2	3.8±0.0	64.9±0.6
fraction 4.2	0.8±0.0	1.6±0.0	-	-	13.9±0.2	-	-	-	8.1±0.4	24.4±0.7
sea buckthorn 'Tytti'										
fraction 1.2	-	-	-	-	3.0±0.1	-	-	-	-	3.0±0.1
fraction 2.1	-	-	-	-	11.7±0.1	-	-	-	-	11.7±0.1
fraction 2.2	-	-	-	-	16.1±0.2	-		-	-	16.1±0.2
fraction 3.1	-	-	-	-	10.2±0.1	-	-	-	-	10.2±0.1
fraction 3.2	-	-	-	-	47.5±0.2	-	-	-	-	47.5±0.2
fraction 4.1	1.0±0.0	-	-	-	30.2±0.2	-	-	-	-	31.2±0.2
fraction 4.2	6.6±0.0	2.0±0.0	-	-	9.2±0.0	-	-	-	-	17.8±0.1
sea buckthorn leaf 'Tytti'										
fraction 1.2	-	-	-	0.8±0.1	14.0±0.5	-	-	-	-	14.8±0.6
fraction 2.1	-	-	-	-	24.4±0.6	-	-	-	12.4±0.8	36.7±1.4
fraction 2.2	-	-	-	18.7±0.2	30.7±0.5	-	-	-	1.0±0.0	50.5±0.8
fraction 3.1	-	-	-	12.9±0.1	28.4±0.2	-	-	-	-	41.3±0.3
fraction 3.2	-	-	217.3±1.1	4.3±0.1	62.6±0.4	-	-	-	-	284.1±1.6
fraction 4.1	8.4±0.0	-	189.9±0.5	6.4±0.0	16.1±0.4	-	-	-	6.0±0.3	226.8±1.2
fraction 4.2	32.6±0.1	-	355.5±1.6	6.4±0.0	15.0±0.1	-	-	-	17.1±0.1	426.6±1.9

(**Table 6** continued)

Fraction No.	*Flavan-3-ols*	*Proanthocyanidins*	*Ellagitannins*	*Phenolic acids*	*Flavonol glycosides*	*Flavone glycosides*	*Flavanone glycosides*	*Anthocyanins*	*Others*	*Sum*
saskatoon leaf										
fraction 1.2	-	-	-	166.7±3.1	-	-	-	-	-	166.7±3.1
fraction 2.1	-	-	-	125.9±1.1	-	-	-	-	-	125.9±1.1
fraction 2.2	-	-	-	185.2±1.0	5.9±0.0	-	-	-	-	191.0±1.1
fraction 3.1	-	-	-	90.9±1.5	127.1±2.7	-	-	-	-	218.0±4.1
fraction 3.2	11.5±0.0	-	-	4.8±0.3	455.2±3.6	-	30.3±0.3	-	-	501.8±4.1
fraction 4.1	66.8±0.8	8.6±0.2	-	48.4±0.4	290.4±1.7	-	-	-	-	414.2±3.0
fraction 4.2	6.0±0.5	32.1±0.5	-	24.1±1.5	32.8±0.3	-	-	-	-	95.0±2.8
whitecurrant leaf										
fraction 1.2	-	-	-	42.9±1.4	34.4±1.3	-	-	-	23.0±0.8	100.4±3.5
fraction 2.1	-	-	-	14.7±1.0	14.3±0.2	-	-	-	23.3±0.2	52.4±1.4
fraction 2.2	-	-	-	1.5±0.0	40.3±0.4	1.0±0.0	-	-	21.0±0.5	63.7±0.9
fraction 3.1	-	-	-	1.1±0.0	126.2±2.7	-	-	-	-	127.3±2.7
fraction 3.2	-	-	-	-	240.9±7.0	-	-	-	-	240.9±7.0
fraction 4.1	5.2±0.3	-	-	-	110.9±3.5	6.5±0.1	-	-	-	122.6±3.8
fraction 4.2	9.7±0.3	-	-	-	39.3±2.2	4.2±0.2	-	-	-	53.2±2.6
lingonberry leaf										
fraction 1.2	-	-	-	17.3±0.4	-	-	-	-	708.6±27.3	725.9±27.7
fraction 2.1	-	-	-	11.3±0.4	-	-	-	-	-	11.3±0.4
fraction 2.2	-	-	-	11.3±0.3	-	-	-	-	-	11.3±0.3
fraction 3.1	-	-	-	10.0±0.2	-	-	-	-	-	10.0±0.2
fraction 3.2	-	-	-	52.4±0.7	106.2±1.0	-	-	-	76.0±0.5	234.6±2.1
fraction 4.1	178.1±0.6	-	-	144.1±0.7	398.5±6.1	-	-	-	-	720.8±7.4
fraction 4.2	55.5±1.0	46.4±0.2	-	1.1±0.1	149.8±2.8	-	-	-	-	252.8±4.1
hawthorn leaf										
fraction 1.2	-	-	-	59.6±1.0	-	-	-	-	-	59.6±1.0
fraction 2.1	-	-	-	64.5±0.3	-	-	-	-	7.2±0.0	71.7±0.3
fraction 2.2	-	-	-	73.7±0.7	-	-	26.1±0.2	-	2.1±0.0	101.9±0.9
fraction 3.1	-	-	-	20.3±0.1	11.6±0.1	0.3±0.0	81.2±0.3	-	6.8±0.1	120.1±0.5
fraction 3.2	2.9±0.0	-	-	-	76.7±0.2	1.4±0.0	-	-	3.2±0.0	84.2±0.3
fraction 4.1	27.2±0.2	6.5±0.1	-	-	112.9±1.0	8.3±0.1	-	-	8.3±0.2	163.2±1.5
fraction 4.2	3.4±0.0	26.4±0.4	-	-	102.0±0.7	-	-	-	0.8±0.0	132.6±1.2

5.1.3 Phenolic composition in berries of blackcurrant cultivars (Study IV)

The sum content of phenolics was 598–2798 mg/100 g in the black-fruited cultivars and 47–104 mg/100 g in the green ones of *Ribes nigrum* (**Table 7**). The low content of total phenolics in green cultivars was due to the absence of anthocyanins. Anthocyanins are the dominating groups of the phenolics. The berries of black cultivars contained 1501 mg/100 g DW of total anthocyanins on average. Delphinidin and cyanidin derivatives formed the major anthocyanins in blackcurrants, accounting for 34–66 % and 31–52 % of sum content of phenolics, respectively. The total content of flavonols ranged from 18 to 60 mg/100 mg DW, representing 37–39 % of the sum content of phenolics in green-fruited cultivars, but only 1–6 % in black ones. Deviation in flavonol profiles was also found between black- and green-fruited cultivars. Myricetin derivatives were the majority of flavonols in the black samples, whereas quercetin glycosides were more abundant in the green cultivars. Flavan-3-ol monomers were quantified at a total amount of 10–20 mg/100 g DW. Among the phenolic acid derivatives, the main components were coumaric acid derivatives (47–74 % of total phenolic acids) in the most of cultivars, followed by those of caffeic (17–40 %) and ferulic acids (9–20 %). Nevertheless, the cultivars ‘Ben Tron’ and ‘Joniniai’ contained more the derivatives of caffeic acid were and less of coumaric acid in the samples of two years studied.

Table 7. Concentrations of the major phenolic compounds (mg/100 g DW) in the fruits of blackcurrant cultivars. Adapted from supporting information of the original publication **IV** (Tian, Laaksonen, Haikonen, Vanag, Ejaz, Linderborg, Karhu, & Yang, 2019)

Cultivars	***Anthocyanins***		***Flavonol glycosides***		***Phenolic acids***		***Flavan-3-ols***		***Sum***	
	2014	***2015***	***2014***	***2015***	***2014***	***2015***	***2014***	***2015***	***2014***	***2015***
Ben Dorain	2125.1±64.4	1733.7±70.7	37.4±3.9	39.4±2.7	14.6±0.4	21.5±0.5	13.1±0.7	15.7±0.4	2192.0±67.2	1812.0±67.4
Ben Gairn	1302.8±295.3	1747.5±307.3	19.4±1.5	33.5±1.8	11.3±0.2	20.3±1.2	11.7±0.5	16.0±0.8	1347.6±294.3	1819.1±303.7
Ben Hope	2532.1±34.6	1320.0±63.7	43.4±0.3	39.0±0.8	21.6±2.3	31.5±2.0	17.0±1.2	17.5±1.1	2616.3±37.2	1410.2±65.8
Ben Starav	1861.8±170.4	1369.0±51.4	29.3±1.0	30.0±2.9	13.4±0.6	17.4±0.9	11.5±0.3	12.8±0.8	1918.2±170.4	1431.9±55.9
Ben Tirran	1625.5±292.4	1533.0±21.5	34.7±2.2	43.0±0.6	22.6±1.2	37.2±2.6	18.0±0.9	23.0±1.4	1703.9±291.2	1639.2±20.0
Ben Tron	2357.1±301.1	2702.6±123.0	54.3±2.3	48.6±2.6	23.3±0.5	24.2±0.1	20.1±0.4	20.2±0.6	2457.9±303.5	2798.6±124.1
S 18/2/23	531.8±78.6	662.6±55.9	29.5±2.7	33.2±3,4	19.2±0.7	31.2±2.5	15.8±0.4	15.3±0.5	598.4±80.2	744.9±55.3
Ben Finlay	1296.3±45.6	2038.0±193.6	32.6±2.5	59.5±4.5	21.4±0.4	29.8±2.0	14.6±0.2	17.3±0.9	1364.9±48.8	2144.6±186.3
9154-3	1169.6±212.6	650.5±35.7	30.5±1.0	45.7±2.7	16.9±0.3	28.8±1.4	12.7±0.3	17.2±1.0	1231.7±211.7	744.9±39.8
Almiai	1511.6±109.2	1254.1±85.4	29.1±1.2	43.2±3.8	12.4±0.5	24.3±3.1	10.2±0.5	18.0±2.1	1565.6±110.1	1343.7±78.3
Dainiai	1312.0±215.9	1886.9±241.0	46.2±5.7	50.5±1.0	18.3±1.5	19.0±0.6	12.8±0.5	15.1±0.2	1393.0±218.8	1974.9±240.1
Gagatai	2248.1±224.7	1635.6±101.8	52.9±3.3	43.3±1.6	15.5±0.8	21.1±1.2	14.2±0.4	16.2±0.3	2333.0±223.8	1718.9±100.0
Joniniai	810.0±82.3	1070.1±80.4	29.2±2.5	47.3±3.2	10.1±0.6	13.6±0.4	13.1±0,9	16.0±1.4	865.7±78.9	1150.6±85.5
Tauriai	1049.2±49.6	847.1±52.9	20.8±0.5	25.1±1.7	11.4±0.5	11.5±0.6	11.1±0.7	12.2±0.5	1094.5±50.6	898.1±50.6
Mara	2638.4±55.7	949.2±73.0	41.0±2.0	46.4±5.2	8.5±0.6	8.4±0.8	12.1±0.8	13.6±0.6	2701.6±55.2	1020.2±78.7
Marski	2552.8±34.6	1545.3±603.7	41.8±2.2	37.7±1.4	10.9±0.4	15.2±0.4	14.1±0.2	14.2±1.1	2623.0±36.9	1615.9±600.9
Mikael	1297.1±33.1	1138.2±39.1	40.7±3.7	38.3±2.2	14.6±0.5	17.4±1.3	13.7±1.0	13.1±0.6	1370.8±28.4	1210.9±42.1
Mortti	1074.4±27.6	813.4±179.2	18.0±1.3	33.9±1.8	11.1±0.5	25.0±1.5	10.2±0.4	17.0±0.4	1115.5±26.3	891.2±181.3
Vilma (green)	-	-	19.3±3.1	40.9±5.7	15.7±1.1	39.2±3.3	13.5±1.4	20.4±2.1	50.7±4.6	103.9±11.4
Venny (green)	-	-	17.5±2.1	24.3±2.4	15.6±1.8	26.0±2.6	11.9±1.4	13.1±1.2	46.9 ±4.7	65.6±4.2
Tisel	943.4±49.7	1344.7±100.4	29.4±1.1	39.2±1.9	9.3±0.2	11.3±0.7	10.1±0.1	11.5±0.4	995.3±51.2	1410.2±103.5

5.2 Anti-oxidative activities of Finnish berry species

5.2.1 Anti-oxidative activity of phenolic extracts (Study II)

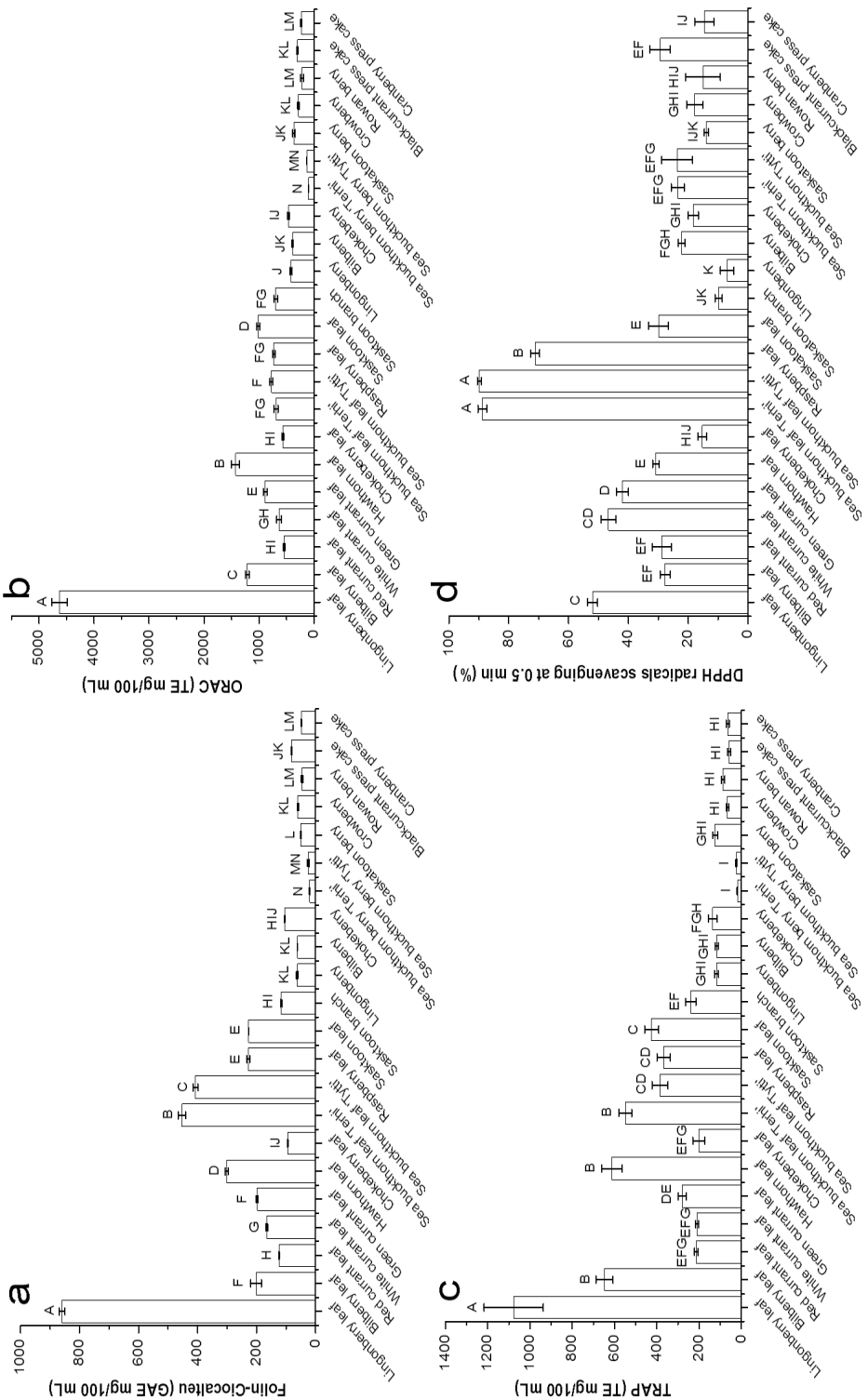
Figure 3 shows the *in vitro* anti-oxidative activity of different extracts of berry plants. Folin-Ciocalteu results were generally in agreement with the results measured by ORAC and TRAP assays. The leaf extracts had higher values of Folin-Ciocalteu reaction than the extracts from berries and branches (**Figure 3a**), reflecting the ability of electron-transferring. The lingonberry leaf extract showed the highest value (860 GAE mg/100 mL), followed by two leaf extracts of sea buckthorn leaves, 'Terhi' and 'Tytti' (407–453). The strongest ability among the berry extracts was found in chokeberry (105). In contrast, the value of the sea buckthorn berry extracts ranged only from 21 to 25 GAE mg/100 mL.

ORAC results suggested that the leaf extracts were better peroxyl-radical scavengers than the berry extracts and the Saskatoon branch extract (**Figure 3b**). The extracts of lingonberry leaf had the best ORAC activity of 4627 TE mg/100 mL. The phenolic extracts of hawthorn leaf (1427 TE mg/100 mL), bilberry leaf (1213), and Saskatoon leaf (1015) were also potent hydrogen donators.

The corresponding berry extracts generally exhibited a lower capacity of quenching peroxyl-radicals compared with the leaf extracts. The extracts of chokeberries, lingonberries and bilberries had the highest ORAC values of 464, 420, and 391 TE mg/100 mL, respectively. The ORAC capacity of the berry extracts of sea buckthorn were 101–130 TE mg/100 mL.

In accordance with ORAC assay, the extract of lingonberry leaf presented a remarkably high TRAP capacity of (1077 TE mg/100 mL), followed by bilberry leaf (648), hawthorn leaf (613), sea buckthorn leaf ('Terhi', 549), and Saskatoon leaf (424). Among the berry extracts, higher TRAP results were also shown in the chokeberry (136 TE mg/100 mL), lingonberry (117), and bilberry (116), which were abundant in anthocyanins. The lowest activities were, again, shown in the extracts of sea buckthorn berries (**Figure 3c**).

Stronger electron-transferring ability of the extracts of leaves was also detected in scavenging DPPH radicals, compared to the berries (**Figure 3d-g**). Over 80 % of DPPH radicals were trapped by most of the leaf extracts within 10 min. With the two sea buckthorn leaf extracts, almost 90 % radicals were trapped in the first 30 s of analysis. Nevertheless, the chokeberry leaf extract succeeded to scavenge about 60 % of DPPH radicals during the measurement. The extracts of chokeberry and blackcurrant press cake captured 80 % DPPH radicals in 10 mins, but the scavenging ability of other berry extracts was lower (30–50 %).


a
Folin-Ciocalteu (GAE mg/100 mL)
b
ORAC (TE mg/100 mL)
c
TRAP (TE mg/100 mL)
d
DPPH radicals scavenging at 0.5 min (%)
Lingonberry leaf
Bilberry leaf
Red currant leaf
White currant leaf
Green currant leaf
Hawthorn leaf
Chokeberry leaf
Sea buckthorn leaf 'Tehti'
Raspberry leaf 'Yykti'
Saskatoon branch
Lingonberry
Bilberry
Chokeberry
Sea buckthorn berry 'Tehti'
Saskatoon berry 'Yykti'
Crowberry
Rowan berry
Blackcurrant press cake
Cranberry press cake

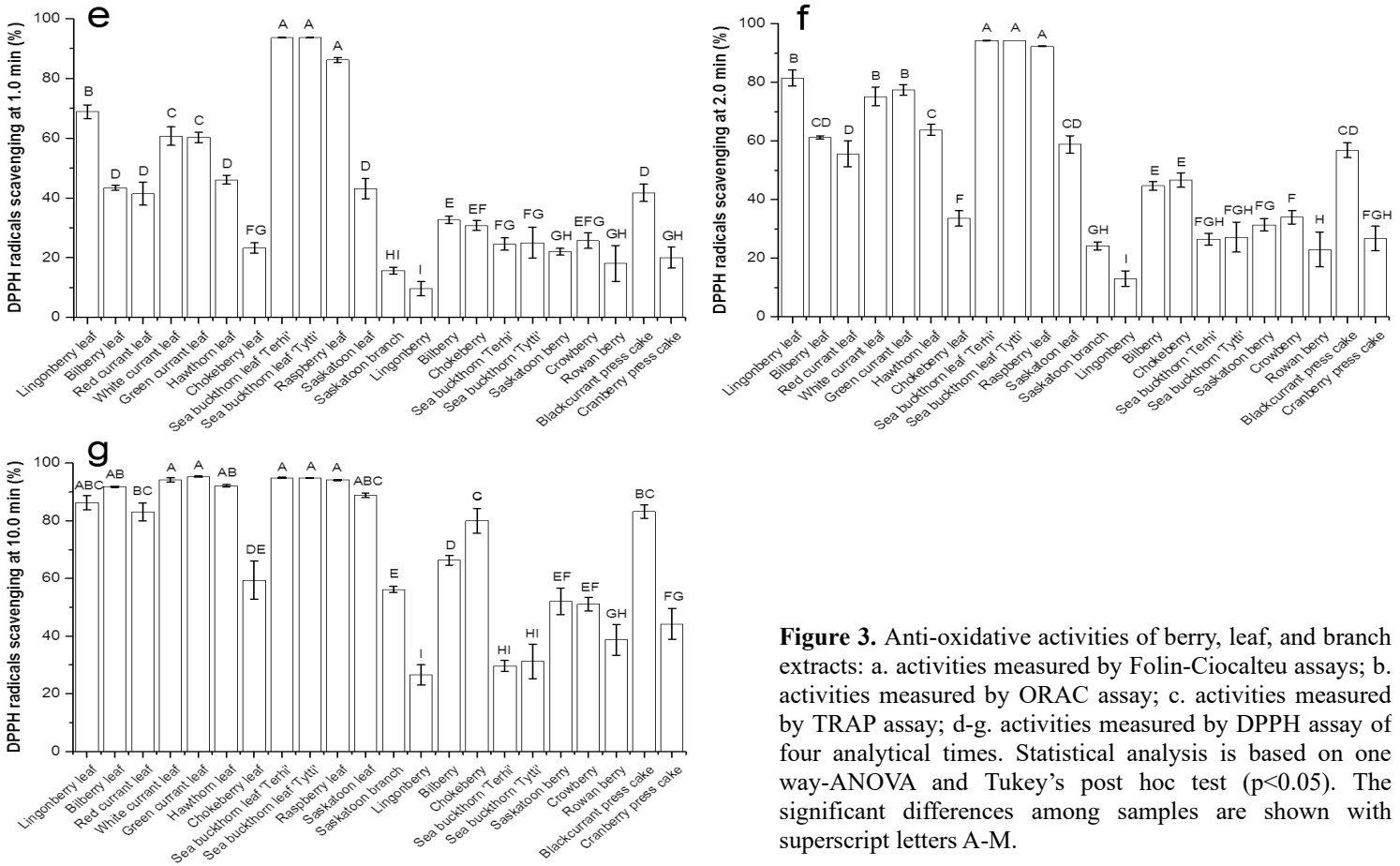


Figure 3. Anti-oxidative activities of berry, leaf, and branch extracts: a. activities measured by Folin-Ciocalteu assays; b. activities measured by ORAC assay; c. activities measured by TRAP assay; d-g. activities measured by DPPH assay of four analytical times. Statistical analysis is based on one way-ANOVA and Tukey's post hoc test ($p<0.05$). The significant differences among samples are shown with superscript letters A-M.

5.2.2 Anti-oxidative activity of fractions of phenolic extracts (Study III)

ORAC activity of berry fractions was generally in the proportion with the sum content of phenolic compounds measured by HPLC (**Table 8**). Chokeberry exhibited the strongest anti-oxidative activity in the fraction 3.2 (17 TE μmol/mL), whereas the highest ORAC value of crowberry was found in the fraction 3.1 (36 TE μmol/mL). Both fractions contained the highest levels of total anthocyanins among all fractions of the corresponding extracts, indicating anthocyanins to be potent inhibitors against peroxyl-radicals. Also, the absence of these compounds might have been the reason of the low capacity of donating hydrogen, such as the berry fractions of sea buckthorn. For the leaf fractions, hawthorn leaf presented the best abilities of transferring hydrogen atom in the fractions 4.1 and 4.2, where flavonols and flavan-3-ols were the dominant phenolics. For lingonberry leaf extract, *β*-*p*-arbutin was abundant in the fraction 1.2, exhibiting also the highest ORAC value (226 TE μmol/mL). The second strongest activity was found in the fraction 4.1(111 TE μmol/mL), likely due to the presence of glycosylquercetins, catechin, and 2-*O*-caffeoyl-*β*-*p*-arbutin. For the leaves of Saskatoon, sea buckthorn, and whitecurrant, unidentified tannins were responsible for strong activities in the fraction 6. Additionally, glycosylated flavonols, primarily as quercetins, correlated to strong activities in the fraction 3.2 of whitecurrant leaf.

Table 8. ORAC activities of the fractions from berry and leaf extracts. Reprinted from the original publication **III** (Tian, Liimatainen, Puganen, Alakomi, Sinkkonen, & Yang, 2018) with permission from Elsevier.

Fraction No.	***ORAC*** *(TE μmol/mL)*		
	chokeberry	***crowberry***	***sea buckthorn***
1.2	8.3±2.0	13.4±1.4	5.1±1.5
2.1	12.5±2.7	8.1±1.5	4.6±1.3
2.2	9.3±2.1	18.1±2.4	3.3±1.3
3.1	6.6±2.1	35.8±4.5	4.0±1.0
3.2	17.3±2.1	11.9±2.5	10.0±1.9
4.1	6.6±1.6	10.8±2.2	9.3±1.3
4.2	6.4±1.0	13.9±2.2	9.5±1.6
5	4.1±1.6	15.1±2.6	6.6±1.4
6	13.2±2.5	27.2±2.3	18.5±2.3
7	4.6±0.9	5.2±1.0	6.4±1.3

(**Table 8** continued)

Fraction No.	***ORAC*** *(TE μmol/mL)*				
	hawthorn leaf	***lingonberry leaf***	***saskatoon leaf***	***sea buckthorn leaf***	***whitecurrant leaf***
1.2	29.9±2.1	226.3±4.2	42.6±1.7	20.7±2.5	23.8±1.6
2.1	22.2±1.5	22.7±1.0	27.4±1.8	17.8±2.8	12.0±0.8
2.2	21.6±1.8	27.7±3.5	36.7±2.5	15.8±2.2	14.7±1.1
3.1	18.1±0.6	14.6±0.6	38.8±0.8	17.6±0.8	25.5±0.7
3.2	24.1±1.9	39.0±2.4	53.6±3.9	22.1±3.6	32.3±1.0
4.1	39.1±2.4	111.3±5.1	68.0±1.2	15.3±2.3	24.5±0.9
4.2	34.9±2.2	54.4±4.0	38.6±2.2	29.6±7.7	14.3±1.2
5	19.1±1.8	56.4±4.4	29.3±2.6	24.7±5.1	7.6±0.8
6	20.0±1.7	87.7±4.3	73.9±2.8	79.3±12.2	31.9±0.9
7	5.6±0.8	13.6±1.0	14.7±1.5	48.2±2.8	9.7±0.2

5.2.3 Correlation of phenolic compounds with anti-oxidative activity (Study II and III)

5.2.3.1 Multivariate correlation between phenolics and anti-oxidative activities

For berry extracts, the Folin-Ciocalteu, ORAC and TRAP activities correlated positively with the concentration of glycosylated cyanidin (mainly cyanidin 3-*O*-galactoside, Cy-Gal) and quercetin (quercetin 3-*O*-galactoside, Qu-Gal). Non-flavonoid phenolics, such as phenolic acids, showed moderate contributions with the activities measured by these three assays. Some di- and tri-glycosides of quercetins and isorhamnetins (Is) showed negative correlations with the anti-oxidative capacities. Anthocyanins, mainly cyanidin 3-*O*-glucoside (Cy-Glu) and delphinidin 3-*O*-glucoside (De-Glu), contributed to DPPH radical scavenging activity. Nevertheless, the capacity of DPPH radical scavenging was associated negatively with the contents of catechins, procyanidins, and mono-glycosylquercetins (**Figure 4a**). In the leaf extracts, strong correlations of flavonoids were found with Folin-Ciocalteu, ORAC and TRAP assays owing to the presence of catechins, procyanidins, and quercetin mono-glycosides. Ellagitannins contributed positively to DPPH radical scavenging activity, as did some flavonol di- and tri-glycosides (**Figure 4b**).

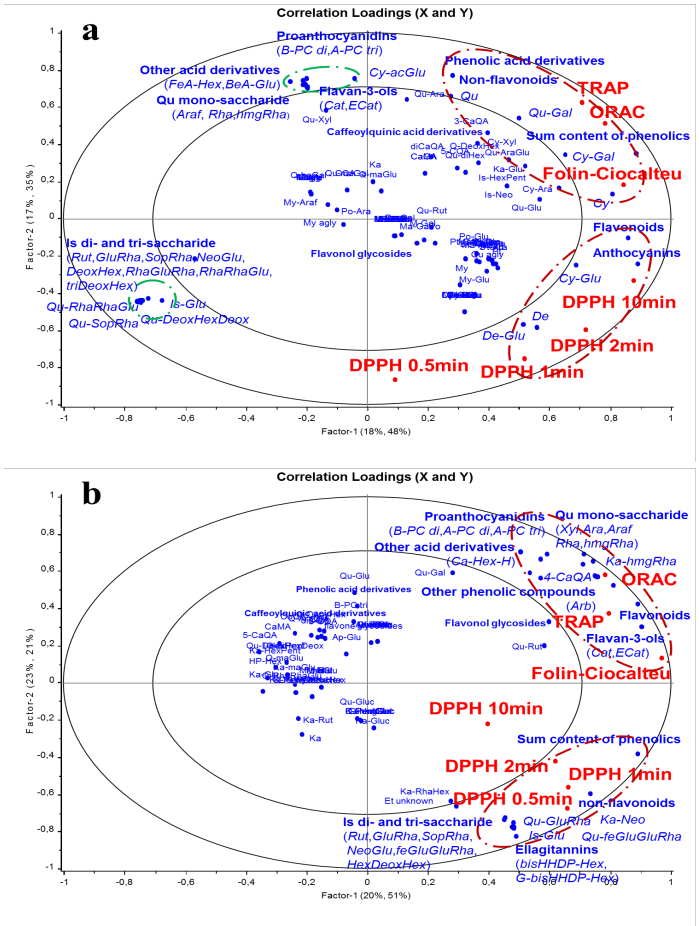
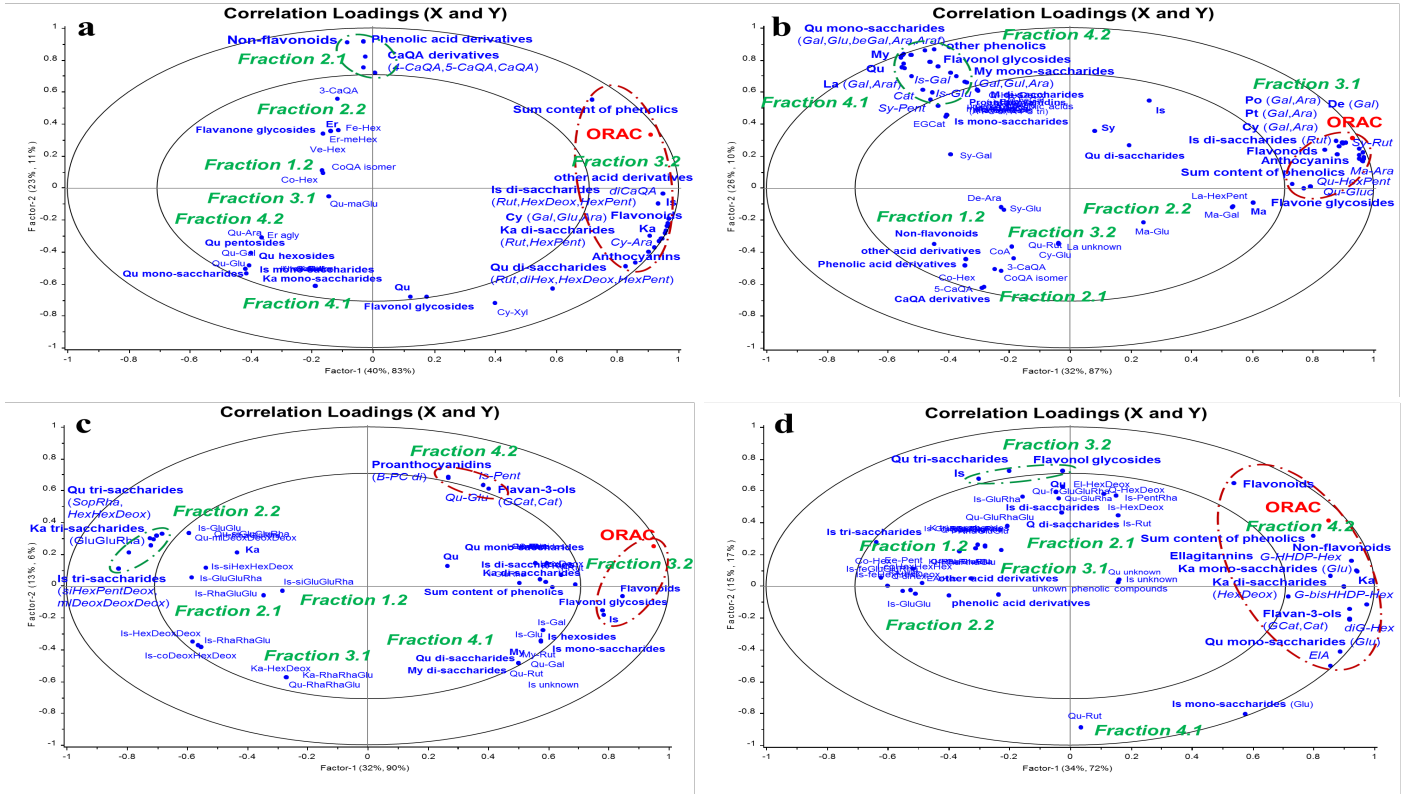


Figure 4. PLS models of Finnish berry plants extracts showing the correlation between chemical variables (X-variables, blue font) and *in vitro* anti-oxidative assays (Y-variables, red font): a. berry and press cake extracts, b. leaf and branch extracts. Reprinted from the original publication **II** (Tian, Puganen, Alakomi, Uusitupa, Saarela, & Yang, 2018) with permission from Elsevier.

Among all the fractions of the extracts of the berry plants studied, ORAC assay was highly associated with the sum content of phenolic compounds (**Figure 5**). Compared with non-flavonoid compounds, flavonoids exhibited stronger correlations with peroxyl-radical scavenging capacity in the berry fractions. Anthocyanins, as the major antioxidants in the dark-skinned berries, were present primarily as cyanidin 3-*O*-galactoside and cyanidin 3-*O*-arabinoside in the fractions of chokeberry; whereas 3-*O*-galactosides of cyanidin, delphinidin, petunidin, and peonidin in the crowberry fractions (**Figure 5a&b**). In contrast, glycosides of flavonols (mostly as isorhamnetins) were the main compounds responsible for ORAC activity in the berry fractions of sea buckthorn (**Figure 5c**). The nature of sugar moieties influenced peroxyl-

radicals scavenging of flavonol glycosides significantly. Strong correlations were found between ORAC activity of chokeberry fractions and certain flavonols with di-saccharides as sugar moieties. Both quercetin and myricetin mono-glycosides showed less correlation with ORAC values of crowberry fractions. Nevertheless, in the berry fractions of sea buckthorn, isorhamnetin bound with tri-saccharides correlated negatively to the capacity of scavenging free radicals; the same was seen for kaempferol and quercetin.

The contribution of phenolic compounds to the capacity of quenching peroxyl-radicals differed among the leaf fractions. In those of sea buckthorn leaves (**Figure 5d**), ORAC activity was ascribed mainly to flavonoids as (+)-catechin, gallocatechin, and kaempferol-hexoside-deoxyhexoside; and ellagitannins as digalloyl-hexoside, galloyl-hexahydroxydiphenoyl-hexoside, and galloyl-bis(hexahydroxydiphenoyl)-hexoside. The main contributors in the fractions of saskatoon leaf were (-)-epicatechin, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, and some quercetin di-glycosides (**Figure 5e**). Strong correlations were found mostly between ORAC values and quercetin 3-*O*-rutinoside, quercetin 3-*O*-rhamnoside-rhamnoside-glucoside, and quercetin-hexoside-pentoside-deoxyhexoside in the whitecurrant leaf fractions (**Figure 5f**). Among lingonberry leaf fractions, *β*-*p*-Arbutin was the major contributors to anti-oxidative effects (**Figure 5g**). The contents of quercetin 3-*O*-galactoside and (-)-epicatechin were associated with the antioxidant activities of hawthorn leaf fractions (**Figure 5h**).


a
Correlation Loadings (X and Y)
Factor-1 (40%, 83%)
Factor-2 (23%, 11%)
Fraction 2.1
Fraction 2.2
Fraction 1.2
Fraction 3.1
Fraction 4.2
Fraction 4.1
Fraction 3.2
ORAC
Non-flavonoids
Phenolic acid derivatives
CaQA derivatives
(t-CaQA,5-CaQA,CaQA)
3-CaQA
Flavanone glycosides
Fe-Hex
Er-meHex
Ve-Hex
CoQA isomer
Co-Hex
Qu-maGlu
Qu-Ara
Qu pentosides
Qu-Gal
Qu hexosides
Qu-Glu
Qu mono-saccharides
Ka mono-saccharides
Qu
Flavonol glycosides
Cy-Xyl
Sum content of phenolics
other acid derivatives
Is di-saccharides
(Rut,HexDeox,HexPent)
dCaQA
Cy (Gal,Glu,Ara)
Flavonoids
Ka di-saccharides
(Rut,HexPent)
Cy-Ara
Anthocyanins
Qu di-saccharides
(Rut,diHex,HexDeox,HexPent)
b
Correlation Loadings (X and Y)
Factor-1 (32%, 87%)
Factor-2 (26%, 10%)
Fraction 4.2
Fraction 4.1
Fraction 3.1
Fraction 1.2
Fraction 2.2
Fraction 3.2
Fraction 2.1
ORAC
Qu mono-saccharides
(Gal,Glu,beGal,Ara,Arap)
other phenolics
My
Flavonol glycosides
Qu
My mono-saccharides
La (Gal,Ara)
Is-Gal
(Gal,Gul,Ara)
Cat
Is-Glu
Di-saccharides
Sy-Pent
EGCat
Is mono-saccharides
Is
Sy
Sy-Gal
Qu di-saccharides
Po (Gal,Ara)
De (Gal)
Pt (Gal,Ara)
Cy (Gal,Ara)
Is di-saccharides (Rut)
Sy-Rut
Flavonoids
Anthocyanins
Sum content of phenolics
Ma-Ara
Qu-HexPent
Qu-Glu
Flavone glycosides
La-HexPent
Ma
Ma-Gal
Ma-Glu
De-Ara
Sy-Glu
Non-flavonoids
other acid derivatives
CoA
Qu-Rut
La unknown
Cy-Glu
Phenolic acid derivatives
3-CaQA
CoQA isomer
Co-Hex
5-CaQA
CaQA derivatives
c
Correlation Loadings (X and Y)
Factor-1 (32%, 90%)
Factor-2 (13%, 6%)
Fraction 4.2
Fraction 2.2
Fraction 1.2
Fraction 2.1
Fraction 3.1
Fraction 4.1
Fraction 3.2
ORAC
Proanthocyanidins
(B-PC di)
Is-Pent
Flavan-3-ols
(GCat,Cat)
Qu-Glu
Qu tri-saccharides
(SopRha, HexHexDeox)
Ka tri-saccharides
(BGluGluRha)
Is tri-saccharides
(diHexPentDeox, mDeoxDeoxDeox)
Is-GluGlu
Is-siHexHexDeox
Ka
Is-GluGluRha
Is-siGluGluRha
Is-RhaGluGlu
Is-HexDeoxHexDeox
Is-RhaRhaGlu
Is-coDeoxHexDeox
Ka-HexDeox
Ka-RhaRhaGlu
Qu-RhaRhaGlu
Qu
Sum content of phenolics
Flavonoids
Flavonol glycosides
Is
Is-Gal
Is-Glu
Is hexosides
Is mono-saccharides
My
My-Rut
Qu-Gal
Qu-Rut
Is unknown
Qu di-saccharides
My di-saccharides
d
Correlation Loadings (X and Y)
Factor-1 (34%, 72%)
Factor-2 (15%, 17%)
Fraction 3.2
Fraction 1.2
Fraction 2.1
Fraction 3.1
Fraction 2.2
Fraction 4.2
Fraction 4.1
ORAC
Qu tri-saccharides
Flavonol glycosides
Is
Flavonoids
Qu
El-HexDeox
Qu-GluGluRha
Qu-GluRha
Is-PentRha
Is-GluRha
Is di-saccharides
Is-HexDeox
Qu-GluRhaGlu
G di-saccharides
Is-Rut
Is tri-saccharides
Sum content of phenolics
Ellagitannins
G-HHDP-Hex
Non-flavonoids
Ka mono-saccharides (Glu)
Ka di-saccharides
(HexDeox)
G-bisHHDP-Hex
Ka
other acid derivatives
Qu unknown
Is unknown
unkown phenolic compounds
Is-GluGlu
phenolic acid derivatives
Flavan-3-ols
(BCat,Cat)
diG-Hex
Qu mono-saccharides (Glu)
EtA
Is mono-saccharides (Glu)
Qu-Rut

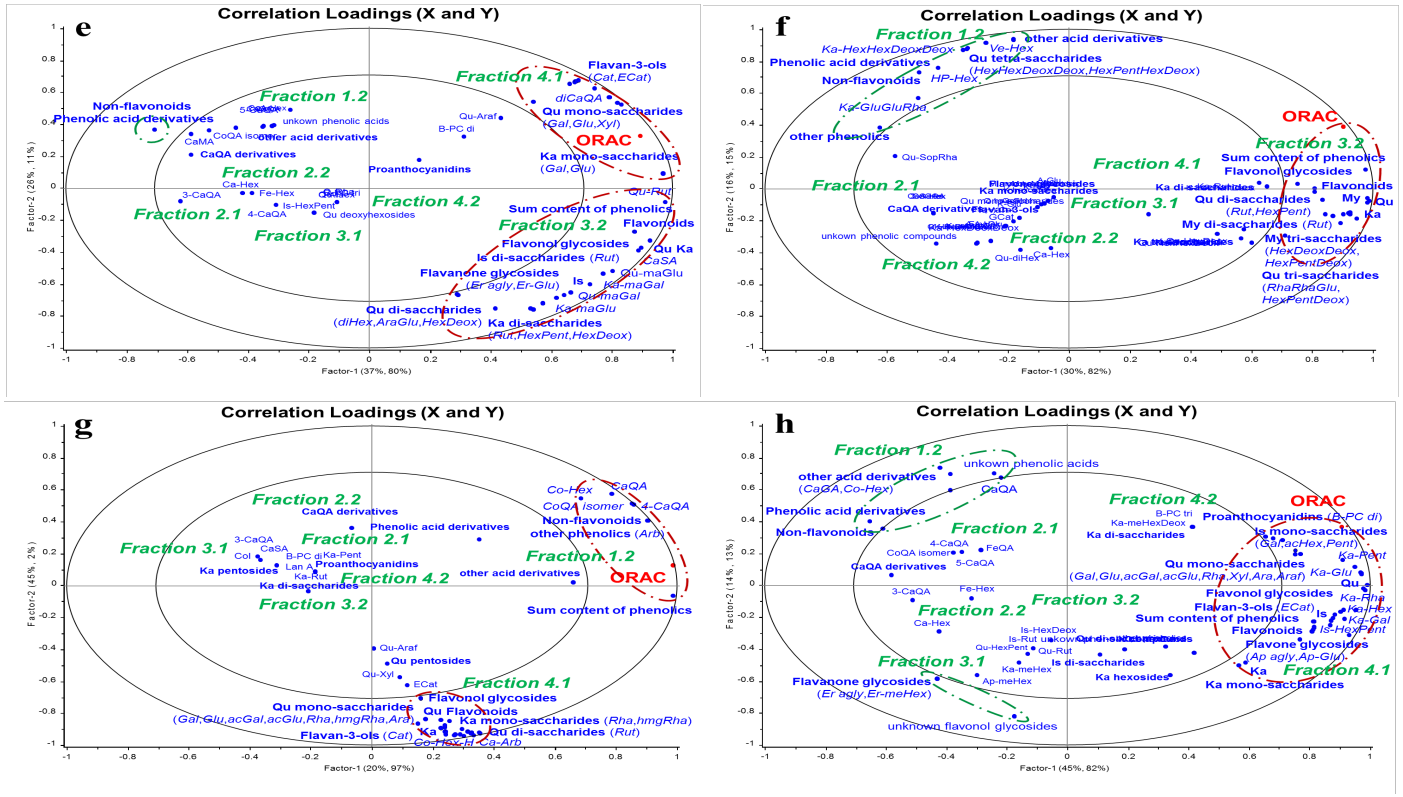


Figure 5. PLS plots of berry and leaf fractions showing the correlation between phenolic composition (blue font) and ORAC assay (red font) in seven fractions (green italic font): (a) chokeberry; (b) crowberry; (c) sea buckthorn; (d) sea buckthorn leaf; (e) saskatoon leaf; (f) whitecurrant leaf; (g) lingonberry leaf; (h) hawthorn leaf. Reprinted from the original publication **III** (Tian, Liimatainen, Puganen, Alakomi, Sinkkonen, & Yang, 2018) with permission from Elsevier.

5.2.3.2 Bivariate correlation between phenolics and anti-oxidative activities

Among all extracts, the sum content of phenolic compounds exhibited the strongest correlation with anti-oxidative activity of Folin-Ciocalteu but weaker association with the activities measured by other assays (**Table 9**). The phenomenon was highly dependent on the composition of phenolic compounds instead of the total phenolic content. Flavonoids correlated to Folin-Ciocalteu (R = 0.888), ORAC (R = 0.961), and TRAP (R = 0.835) assays more strongly than did the non-flavonoid phenolic compounds. The contribution among flavonoids to activates measured by these three assays was ranked as the following: proanthocyanidins (mostly procyanidin dimers and trimers) > flavan-3-ols ((+)-catechin and (-)-epicatechin) > flavonols (quercetin glycosides). Flavonol glycosides were associated significantly with DPPH radical scavenging mostly due to the content of isorhamnetin (R = 0.733–0.909) and quercetin (R = 0.552–0.571) derivatives. The total content of anthocyanins correlated highly with the activity of quenching DPPH radicals (R = 0.778–0.802). No significant bivariate correlation was established between the content of cyanidins and activities measured by DPPH and TRAP assays, although positive correlations were seen between cyanidins and Folin-Ciocalteu (R = 0.763) and ORAC (R = 0.751) values. Non-flavonoid phenolics showed significant correlations (R = 0.682–0.839) with the anti-oxidative values measured by DPPH assay. The total content of phenolic acid derivatives correlated with TRAP activity (R = 0.520). Moreover, phenolic acid conjugates (excluding caffeoylquinic acids) presented strong correlations with Folin-Ciocalteu (R = 0.672) and ORAC activities (R = 0.707).

The ORAC values of the berry and leaf fractions also correlated significantly with the sum content of phenolics quantified. Nevertheless, both flavonoids and non-flavonoid phenolics contributed mainly to scavenging of peroxyl-radicals. As the major hydrogen-donors in the berry fractions, anthocyanins had the highest correlation coefficient value with ORAC values. The contribution of other flavonoids decreased in the order: flavan-3-ols, glycosides of flavonols, flavanones and flavones. In agreement with the PLS models of fractions (**Figure 5c-f**), the number of sugar moieties may play an essential role on anti-oxidative activities of quercetin glycosides. In the study **III**, mono-glycosylquercetin generally showed a high correlation coefficient with ORAC (R = 0.898), followed by its di-glycosides (R = 0.548) and tri-glycosides (R = 0.620).

The contribution of certain individual phenolic compounds to ORAC activity was investigated in the study **III**. The coefficient value between (+)-catechin and ORAC (R = 0.825) was significantly higher compared to its stereo-isomer, (-)-epicatechin (R = 0.704). A tri-glycoside of quercetin, quercetin 3-*O*-rhamnoside-rhamnoside-glucoside, showed higher coefficient

value than that of quercetin 3-*O*-sophoroside-7-*O*-rhamnoside, suggesting the impact of the glycosylation at 7-OH on the anti-oxidative activities of flavonols. In addition, quercetin 3-*O*-rhamnoside correlated highly with ORAC results (R = 0.963), as well as 3-*O*-arabinoside, 3-*O*-rutinoside, and 3-*O*-galactoside of quercetins. Moreover, the correlation coefficients of anthocyanidin 3-*O*-galactoside with ORAC values were generally ranked in the following order: cyanidin < delphinidin < petunidin < peonidin.

Table 9. Pearson correlation coefficients between phenolics and anti-oxidative activities in the extracts and fractions of berry plants. Adapted from supporting information of the original publication **II** (Tian, Puganen, Alakomi, Uusitupa, Saarela, & Yang, 2018).

	***Electron transfer** (ET)*					***Hydrogen atom transfer** (HAT)*	
	Folin-Ciocalteu	***DPPH***				***ORAC***	***TRAP***
		0.5 min	***1 min***	***2 min***	***10 min***		
Extracts							
sum content of phenolics[a] (n=24)	0.856**	0.838**	0.796**	0.718**	0.517**	0.563**	0.713**
total flavonoids (n=24)	0.888**	0.374	0.467*	0.525**	0.491*	0.961**	0.835**
total non-flavonoids[b] (n=21)	0.682**	0.839**	0.756**	0.644**	0.415	0.308	0.523*
total flavan-3-ols (n=11)	0.907**	0.274	0.322	0.319	0.156	0.944**	0.795**
total proanthocyanidins (n=7)	0.978**	0.848*	0.804*	0.726	0.495	0.975**	0.856*
total phenolic acid derivatives (n=18)	0.267	0.146	0.199	0.241	0.295	0.307	0.520*
caffeoylquinic acid derivatives (n=17)	0.115	0.119	0.187	0.254	0.365	0.141	0.397
other acid derivatives (n=11)	0.672*	0.242	0.218	0.155	0.004	0.707*	0.557
total flavonol glycosides (n=24)	0.781**	0.570**	0.666**	0.714**	0.650**	0.753**	0.764**
quercetin glycosides (n=24)	0.776**	0.394	0.503*	0.571**	0.552**	0.834**	0.785**
isorhamnetin glycosides (n=13)	0.755**	0.909**	0.847**	0.733**	0.414	0.237	0.510
kaempferol glycosides (n=15)	-0.110	0.334	0.421	0.472	0.450	-0.151	-0.109
total anthocyanins (n=8)	0.555	0.665	0.778*	0.802*	0.782*	0.487	0.223
cyanidin glycosides (n=8)	0.763*	0.248	0.456	0.567	0.688	0.751*	0.665
Fractions							
sum content of phenolics (n=224)	-	-	-	-	-	0.921**	-
total flavonoids (n=180)	-	-	-	-	-	0.856**	-
total non-flavonoids (n=168)	-	-	-	-	-	0.839**	-
total flavan-3-ols (n=64)	-	-	-	-	-	0.878**	-
(+)-catechin (n=48)	-	-	-	-	-	0.825**	-
(-)-epicatechin (n=36)	-	-	-	-	-	0.704**	-
total flavonol glycosides (n=168)	-	-	-	-	-	0.805**	-
quercetin glycosides (n=160)	-	-	-	-	-	0.650**	-
quercetin tri-saccharides (n=60)	-	-	-	-	-	0.620**	-
quercetin 3-*O*-sophoroside-7-*O*-rhamnoside (n=32)	-	-	-	-	-	0.359*	-
quercetin 3-*O*-rhamnoside-rhamnoside-glucoside (n=28)	-	-	-	-	-	0.659**	-
quercetin di-saccharides (n=104)	-	-	-	-	-	0.548**	-
quercetin 3-*O*-rutinoside (n=56)	-	-	-	-	-	0.840**	-
quercetin mono-saccharides (n=88)	-	-	-	-	-	0.898**	-
quercetin 3-*O*-galactoside (n=48)	-	-	-	-	-	0.818**	-

(**Table 9** continued)

	Electron transfer *(ET)*					***Hydrogen atom transfer*** *(HAT)*	
	Folin-Ciocalteu	***DPPH***				***ORAC***	***TRAP***
		0.5 min	***1 min***	***2 min***	***10 min***		
quercetin 3-*O*-glucoside (n=76)	-	-	-	-	-	0.664**	-
quercetin 3-*O*-rhamnoside (n=20)	-	-	-	-	-	0.963**	-
quercetin 3-*O*-arabinoside (n=32)	-	-	-	-	-	0.859**	-
quercetin 3-*O*-arabinofuranoside (n=36)	-	-	-	-	-	0.514**	-
total flavone glycosides (n=36)	-	-	-	-	-	0.425**	-
total flavanone glycosides (n=24)	-	-	-	-	-	0.650**	-
total anthocyanins (n=36)	-	-	-	-	-	0.965**	-
delphinidin 3-*O*-galactoside (n=20)	-	-	-	-	-	0.965**	-
cyanidin 3-*O*-galactoside (n=36)	-	-	-	-	-	0.821**	-
cyanidin 3-*O*-glucoside (n=20)	-	-	-	-	-	0.858**	-
petunidin 3-*O*-galactoside (n=20)	-	-	-	-	-	0.968**	-
peonidin 3-*O*-galactoside (n=20)	-	-	-	-	-	0.984**	-

[a] the sum of concentration of phenolics analyzed by HPLC-DAD; [b] non-flavonoids comprised ellagitannins, phenolic acid derivatives, and other phenolic compounds;
** correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed).

5.3 Anti-bacterial activities of Finnish berry species

5.3.1 Anti-bacterial activity of phenolic extracts (Study II)

Targeted bacteria showed different sensitivity to the leaf and fruit extracts (**Table 10**). Clear inhibitory effect against *E. coli* was found when the extracts were added to 20 µL (in 300 µL culture medium). Both leaf and branch extracts of saskatoon showed 75 % and 68 % inhibition on growth of *E. coli* strains, respectively. The press cakes of blackcurrant and cranberry presented the best anti-*E. coli* effects (67 % for each) among the berry extracts. At the low concentration (10 µL), the leaf extracts of bilberry and chokeberry showed no inhibition against *E. coli*, and limited effects were shown in the two sea buckthorn berry extracts at the same dose. Both being Gram-negative bacteria, *S. enterica* sv. Typhimurium showed higher sensitivity to the extracts than *E. coli* strains. The extracts inhibited 33–54 % of the growth of *S. enterica* at 10 µL of addition, and 34–100 % at 20 µL. The inhibitory effect against *S. enterica* was generally in proportion with the concentration of extracts used in culture medium; however, the growth inhibition was less than 40 % when the berry extract of sea buckthorn cultivar 'Terhi' were added at either of the two concentrations.

Among Gram-positive bacteria, *S. aureus* was sensitive to the leaf extracts of two sea buckthorn cultivars (99–100 %), lingonberry (92 %), and hawthorn (87 %) at the low dose. This may have been due to ellagitannins, flavan-3-ols, and proanthocyanidins as the dominant phenolic compounds present in the corresponding extracts. Nevertheless, berry extracts of bilberry and Saskatoon inhibited only 30 % of the growth of *S. aureus* when used at high level. For *Listeria monocytogenes*, the leaf extracts of sea buckthorn and raspberry, all containing high content of ellagitannins, had stronger efficacy than other extracts at the low dose; weaker inhibition was seen in bilberries, lingonberries, and sea buckthorn berries ('Terhi'), used at high dose. *B. cereus* strains showed strong tolerance to some extract such as those from lingonberries, bilberries, bilberry leaves, saskatoon berries, and rowanberries. Strong efficacy against *B. cereus* was observed in the extracts of sea buckthorn leaves (94–98 %), hawthorn leaves (95 %), and lingonberry leaves (90 %).

Table 10. Antibacterial activities (growth inhibition %) of berry plant extracts. Reprinted from the original publication **II** (Tian, Puganen, Alakomi, Uusitupa, Saarela, & Yang, 2018) with permission from Elsevier.

Sample name	***E. coli***		***S. aureus***		***L. monocytogenes***		***B. cereus***		***S. enterica* sv. Typhimurium**	
	10 µL	***20 µL***	***10 µL***	***20 µL***	***10 µL***	***20 µL***	***10 µL***	***20 µL***	***10 µL***	***20 µL***
Berry extracts										
lingonberry	23±1	43±3	43±4	90±2	53±1	92±1	-3±2	-3±0	45±1	84±17
bilberry	38±3	58±2	11±2	33±3	25±1	77±2	-5±2	-4±1	-	-
chokeberry	40±2	59±4	24±1	74±2	54±13	99±3	-	82±20	-	-
sea buckthorn 'Terhi'	1±0	32±1	14±6	48±3	6±1	43±3	-6±2	27±3	33±5	34±0
sea buckthorn 'Tytti'	4±0	42±1	21±2	64±2	45±3	92±1	-3±1	90±2	35±1	98±0
saskatoon berry	42±4	57±5	16±0	31±6	17±3	74±1	-7±0	-6±2	-	-
crowberry	14±2	33±1	36±2	66±3	25±1	84±0	-3±1	89±4	45±0	77±6
rowanberry	22±1	47±3	16±3	61±3	18±1	72±2	-4±1	-4±1	44±0	50±1
Press cake extracts										
blackcurrant	43±1	67±2	55±7	100±0	57±4	100±0	6±7	77±37	-	-
cranberry	38±3	67±4	33±2	97±1	56±8	100±0	-1±2	89±14	-	-
Leaf extracts										
lingonberry	26±2	50±3	92±1	100±0	54±2	37±1	90±3	100±0	54±5	71±4
bilberry	-2±0	16±3	28±3	40±2	-1±0	43±1	-7±3	6±1	40±0	58±8
redcurrant	8±2	36±3	54±4	77±4	6±2	83±2	1±1	26±2	41±1	67±4
whitecurrant	12±1	39±1	49±5	91±3	44±3	73±3	-3±1	90±3	50±2	78±12
hawthorn	20±1	40±2	87±3	100±0	53±1	100±0	95±4	100±0	37±8	86±4
chokeberry	0±0	23±2	53±4	72±4	9±4	89±1	1±1	98±1	40±2	68±15
sea buckthorn 'Terhi'	24±4	55±5	99±1	100±0	100±0	100±0	98±2	100±0	50±1	100±5
sea buckthorn 'Tytti'	26±4	47±3	100±0	100±0	100±0	100±0	94±1	100±0	49±1	87±12
raspberry	16±4	43±7	61±5	95±3	80±2	100±0	25±3	96±2	48±3	81±5
saskatoon	53±3	75±4	68±6	100±0	71±7	100±0	67±21	89±16	-	-
Branch extracts										
saskatoon	38±3	68±4	56±2	100±0	66±3	100±0	4±5	84±19	-	-

5.3.2 Anti-bacterial activity of fractions of phenolic extracts (Study III)

Anti-bacterial activity of fractions from selected berry and leaf extracts were evaluated only with the strains of *S. aureus* and *E. coli*, representing Gram positive and Gram negative bacteria, respectively. **Table 11** shows that inhibitory effects against both targeted microbes were observed in most of the fractions applied at lower concentration (10 µL in 300 µL of media). Fraction 6 of sea buckthorn berry ('Tytti') extract represented the highest growth inhibition (88 %) against *S. aureus* at low dose. The second strongest effect was found in the fraction 6 of the chokeberry extract (87 %). This may have been due to the abundance of ellagitannins in these two fractions. Nevertheless, anti-bacterial activity of ellagitannins might be associated with their composition, since the strains exhibited strong resistance to fraction 7 of the sea buckthorn leaf extract (12 %) when 20 µL of volume was added in the cultivation media, as well as to fraction 6 of lingonberry leaf (37 %). Additionally, weaker growth inhibition was shown in the fraction 4.1 of sea buckthorn berry extract (13–15 % at both dosage levels).

Compared to *S. aureus*, *E. coli* generally had higher resistance to the fractions used at low dosage levels, which was in agreement with the results from evaluation of the extracts. This may be owing to the outer membrane in Gram-negative organisms that restrict the diffusion of hydrophobic compounds. Anti-*E. coli* effect was enhanced with increasing doses in most of the fractions; however, the fraction 6 of Saskatoon leaf and whitecurrant leaf had no inhibition observed at either dosage level. The fraction 7 from these two extracts inhibited only 10–20 % of the growth of *E. coli* strains.

Table 11. Growth inhibition (%) of *Staphylococcus aureus* and *Escherichia coli* induced by leaf and fruit fractions of berry species. Reprinted from the original publication **III** (Tian, Liimatainen, Puganen, Alakomi, Sinkkonen, & Yang, 2018) with permission from Elsevier.

Fraction No.	***Amount (µL/300 µL)***	***Growth inhibition***							
		chokeberry	***crowberry***	***sea buckthorn***	***hawthorn leaf***	***lingonberry leaf***	***saskatoon leaf***	***sea buckthorn leaf***	***whitecurrant leaf***
S. aureus									
1.2	10	61±5	48±5	72±1	56±2	46±6	54±2	67±5	53±12
	20	88±2	73±1	89±0	73±10	85±0	73±3	84±0	78±4
2.1	10	49±3	43±2	38±0	67±6	53±5	34±0	65±1	65±3
	20	64±2	77±1	70±0	84±1	75±5	67±4	78±4	87±2
2.2	10	46±1	38±3	30±6	51±2	43±5	28±3	51±9	74±23
	20	67±1	66±6	75±4	67±19	77±1	78±12	83±2	81±4
3.1	10	56±0	47±9	44±3	63±16	49±5	57±1	64±0	67±1
	20	67±2	70±6	74±1	71±2	69±3	74±2	86±1	82±1
3.2	10	52±5	62±0	50±1	56±3	38±0	49±3	62±0	69±0
	20	64±12	70±11	81±9	79±1	72±2	70±24	85±0	83±14
4.1	10	57±1	64±5	13±4	68±1	60±1	71±1	65±4	71±1
	20	72±1	75±0	15±4	80±0	88±6	89±3	87±0	73±0
4.2	10	56±2	60±4	59±1	46±3	65±6	52±5	70±3	70±2
	20	74±1	67±0	79±5	76±4	84±3	88±6	90±0	78±2
5	10	54±0	67±6	48±13	67±3	68±2	41±17	68±11	72±4
	20	66±0	80±0	88±0	77±1	73±1	84±5	73±20	79±9
6	10	87±2	36	88±0	61±6	37±9	56±7	67±2	62±2
	20	85±3	65±12	85±2	75±1	37	98±3	76±0	60
7	10	55±6	59±2	66±2	63±10	63±4	26±3	17±3	65±2
	20	77±1	68±3	76±0	72±0	70±0	67±5	12±4	86±7

(**Table 11** continued)

Fraction No.	*Amount (µL/300 µL)*	*Growth inhibition*							
		chokeberry	*crowberry*	*sea buckthorn*	*hawthorn leaf*	*lingonberry leaf*	*saskatoon leaf*	*sea buckthorn leaf*	*whitecurrant leaf*
E. coli									
1.2	10	53±1	41±19	66±0	46±0	46±3	46±3	36±2	44±2
	20	93±9	89±8	99±0	75±12	78±3	79±4	85±17	92±7
2.1	10	45±4	49±4	34±3	41±2	40±2	35±0	37±1	33±6
	20	83±5	88±0	55±2	50±23	67±1	76±2	82±8	38±9
2.2	10	49±3	40±2	41±2	42±2	40±2	31±2	48±5	36±1
	20	88±3	68±4	74±0	81±6	70±0	77±16	34±18	71±13
3.1	10	48±3	36±5	39±7	35±3	21±15	39±7	44±2	67±39
	20	91±8	55±7	70±1	67±4	92±6	66±9	86±1	69±6
3.2	10	48±1	32±10	42±4	33±2	37±1	34±0	54±0	38±5
	20	76±9	80±5	82±8	63±8	65±1	55±1	86±5	65±1
4.1	10	42±4	34±1	45±6	33±8	38±1	32±1	51±3	33±2
	20	84±1	67±8	52±29	55±4	70±3	39±4	81±11	56±1
4.2	10	40±0	37±6	47±2	37±2	42±2	31±5	58±3	38±1
	20	79±3	77±7	98±2	60±3	89±2	57±6	88±12	60±2
5	10	49±7	31±1	46±1	35±6	54±6	25±0	58±14	34±0
	20	88±14	66±3	74±9	72±4	66±0	61±0	74±4	68±3
6	10	47±2	12±4	32±11	31±0	40±27	0±0	57±2	0±0
	20	63±4	22±13	74±8	56±8	60±4	0±0	49±2	0±0
7	10	34±2	19±10	41±8	33±3	35±0	10±1	n.d.	18±1
	20	71±11	49±2	68±7	73±22	77±10	21±12	n.d.	22±24

5.3.3 Correlation of phenolic compounds with anti-bacterial activity (Study II and III)

Successful PLS regression model was built only between phenolic composition of leaf extracts and the activities against *S. aureus* and *B. cereus*, respectively. In **Figure 6**, the leaf extracts were grouped based on the growth inhibition at low dosage level. The sum content of phenolics correlated strongly to anti-*S. aureus* and anti-*B. cereus* activities. The major inhibitors were di- and tri-glycosylated isorhamnetins, and ellagitannins, as well as kaempferol 3-*O*-neohesperidoside (K-Neo), quercetin 3-*O*-(6-*O*-feruloylglucoside)-glucoside-7-*O*-rhamnoside (Q-feGluGluRha), and quercetin 3-*O*-glucoside-7-*O*-rhamnoside (Q-GluRha). Moderate correlation was found with flavan-3-ols (primarily as (+)-catechin and (-)-epicatechin) and proanthocyanidins (dimers of B-type procyanidin) along factor 1. Yet, the derivatives of phenolic acids were associated negatively with inhibitory effect against these two strains.

Pearson's correlation between phenolics and anti-bacterial activity is presented in **Table 12**. The sum content of phenolics correlated significantly to inhibition of *B. cereus* (R = 0.825), *S. aureus* (R = 0.772), *S. enterica* sv. Typhimurium (R = 0.665), and *L. monocytogenes* (R = 0.609). Both anti-*B. cereus* and anti-*S. aureus* activities exhibited stronger correlation with the content of non-flavonoid phenolic compounds, compared to that of flavonoids in extracts. Among flavonoids, proanthocyanidins (procyanidin dimers and trimers) and glycosylated flavonols (quercetin) showed higher coefficient values of 0.761 and 0.647, respectively, with inhibition against *S. aureus* strains. The high correlation coefficient value of 0.617 suggested quercetin glycosides as a strong inhibitor against the *Bacillus cereus* strain. No significant correlation was found between main groups of phenolics with the inhibitory effect against *E. coli*. The contribution of individual phenolic compounds to anti-bacterial activity could be not determined successfully based on the data in the study **III**.

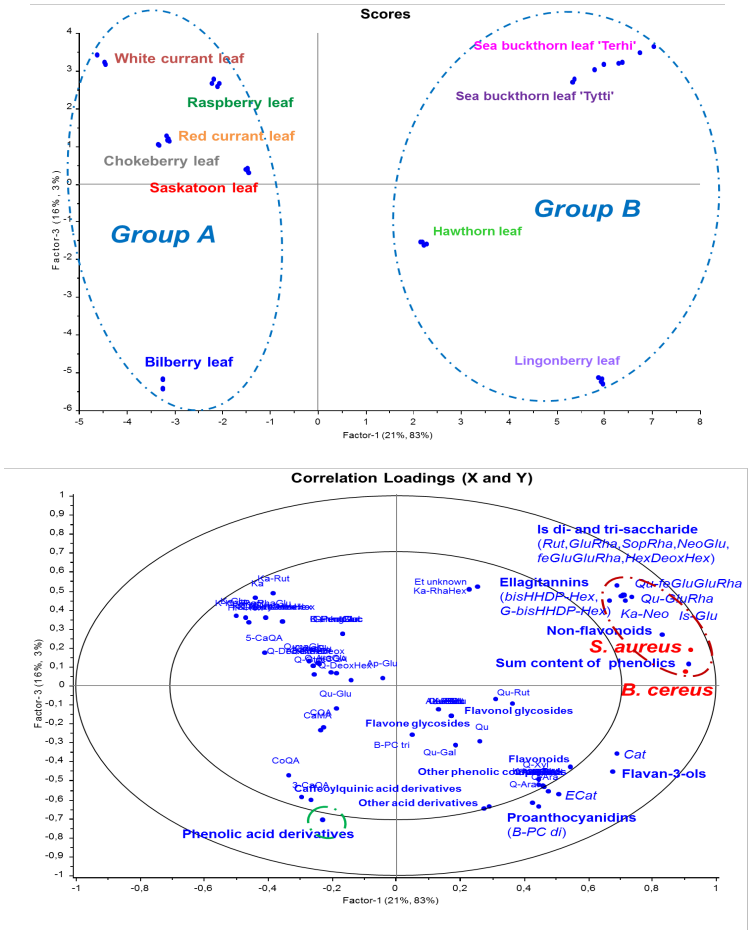


Figure 6. PLS models of Finnish berry plants extracts presenting the correlation between chemical variables (blue font) and activities against *Staphylococcus aureus* and *Bacillus cereus* strains (red font). Reprinted from the original publication **II** (Tian, Puganen, Alakomi, Uusitupa, Saarela, & Yang, 2018) with permission from Elsevier.

Table 12. Pearson correlation coefficients between phenolics and anti-bacterial assays in the berry plant extracts. Reprinted from the original publication **II** (Tian, Puganen, Alakomi, Uusitupa, Saarela, & Yang, 2018) with permission from Elsevier.

	E. coli		***S. aureus***		***L. monocytogenes***		***B. cereus***		***S. enterica* sv. Typhimurium**	
	10 µL	***20 µL***	***10 µL***	***20 µL***	***10 µL***	***20 µL***	***10 µL***	***20 µL***	***10 µL***	***20 µL***
sum content of phenolics[a] (n=22)	0.104	0.116	0.772**	0.420	0.609**	0.052	0.825**	0.393	0.665**(n=15)	0.433(n=15)
total flavonoids (n=22)	0.227	0.190	0.576**	0.406	0.301	-0.133	0.640**	0.357	0.560*(n=15)	0.209(n=15)
total non-flavonoids[b] (n=19)	0.008	0.092	0.696**	0.335	0.594**	0.100	0.725**	0.318	0.552(n=13)	0.497(n=13)
total flavan-3-ols (n=10)	0.124	0.121	0.525	0.355	0.189	-0.483	0.509	0.400	0.594(n=8)	0.058(n=8)
total proanthocyanidins (n=7)	0.172	0.184	0.761*	0.458	0.347	-0.485	0.699	0.675	0.728(n=5)	0.180(n=5)
total phenolic acid derivatives (n=16)	-0.187	-0.289	-0.116	-0.344	-0.237	-0.380	0.083	-0.331	-0.034(n=10)	-0.146(n=10)
caffeoylquinic acid derivatives (n=15)	-0.117	-0.266	-0.179	-0.313	-0.236	-0.239	-0.016	-0.280	-0.158(n=10)	-0.147(n=10)
other acid derivatives (n=10)	0.062	0.012	0.489	0.042	0.097	-0.368	0.571	-0.194	0.402(n=6)	0.083(n=6)
total flavonol glycosides (n=22)	0.019	0.034	0.674**	0.502*	0.336	0.000	0.660**	0.459*	0.561*(n=15)	0.282(n=15)
quercetin glycosides (n=22)	0.100	0.085	0.602**	0.452*	0.247	-0.070	0.617**	0.401	0.503(n=15)	0.175(n=15)
isorhamnetin glycosides (n=12)	-0.114	-0.009	0.547	0.218	0.564	0.126	0.705*	0.170	0.564(n=8)	0.478(n=8)
kaempferol glycosides (n=14)	-0.328	-0.245	-0.089	0.089	0.015	0.130	-0.196	-0.121	0.209(n=10)	0.203(n=10)
total anthocyanins (n=8)	0.320	0.147	-0.142	-0.386	-0.070	0.003	-0.007	0.201	-(n=3)	-(n=3)
cyanidin glycosides (n=8)	0.540	0.279	-0.263	-0.303	0.106	0.148	-0.318	0.153	-(n=3)	-(n=3)

[a] the sum of concentration of phenolics analyzed by HPLC-DAD; [b] non-flavonoids comprised ellagitannins, phenolic acid derivatives, and other phenolic compounds;
** correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed).

5.4 Variation of phenolic profiles among cultivars and growing years (study IV)

Phenolic profiles in berry of blackcurrant exhibited significant variation among different cultivars. The green-fruited cultivars were distinguished from black-fruited ones due to the lower contents of anthocyanins and myricetins in the former ones (**Figure 7a**). The black-fruited cultivars of same origin may share more similarities than those originated from different countries. Suggested by PLS regression models (**Figure 7**), the concentration of phenolic acid derivatives were the main difference among the cultivars originating from Finland, Lithuania, and Scotland. The Scottish cultivars presented a higher total amount of phenolic acid derivatives than the Lithuanian samples, ascribing mostly to 4-*O*-coumaroylglucose (4-Co-Glu), (*E*&*Z*)-coumaroyloxymethylene-glucopyranosyloxy-(*Z*)-butenenitrile (Co-meGlu-B1&2), and 1-*O*-feruloylglucose (1-Fe-Glu). The Finnish black-fruited cultivars contained lower contents of 5-*O*-caffeoylquinic acid (5-CaQA), 1-*O*-caffeoylglucose (1-Ca-Glu), and Co-meGlu-B1&2, compared to Scottish samples. Lithuanian cultivars were richer in 5-CaQA and 3-*O*-coumaroylquinic acid (3-CoQA) than the Finnish black cultivars (**Figure 7b-d**).

The nine samples of Scottish origin were grouped based on the scores plot of PLS regression model. Group A consisted of cultivars ‘Ben Dorain’, ‘Ben Gairn’, ‘Ben Starav’ and ‘Ben Finlay’. Group B included cultivars ‘S 18/2/23’ and ‘9154-3’. Cultivars ‘Ben Hope’, ‘Ben Tirran’, and ‘Ben Tron’ belonged to group C. **Figure 8a** suggested that higher sum-content of phenolic and total anthocyanins were quantified from the cultivars in group A than in those of group B. This was mainly due to the presence of cyanidin 3-*O*-rutinoside (Cy-Rut), delphinidin 3-*O*-rutinoside (De-Rut), and delphinidin 3-*O*-glucoside (De-Glu) at high contents in group A. Cultivars ‘S 18/2/23’ and ‘9154-3’ contained more 4-*O*-caffeoylglucose (4-Ca-Glu) and 4-*O*-coumaroylglucose (4-Co-Glu) than the samples in group A.

Group A was low in flavan-3-ols and the conjugates of caffeic acid and ferulic acid (CaA and FeA) compared to group C. Some minor flavonols were absent in the samples of group C, such as quercetin 3-*O*-galactoside (Qu-Gal), quercetin 3-*O*-arabinoside (Qu-Ara), myricetin 3-*O*-galactoside (My-Gal), and isorhamnetin 3-*O*-(6"-malonyl)-galactoside (Is-maGal) (**Figure 8b**). Compared to group C (**Figure 8c**), group B had lower value of sum content of phenolics, owing to the lower content of anthocyanins and flavonols (myricetin derivatives). Lithuanian cultivars were classified as group A (‘Almiai’, ‘Dainiai’, and ‘Gagatai’) and group B (‘Joniniai’ and ‘Tauriai’). The cultivars in group A correlated highly with delphinidins, cyanidins, myricetins, and ferulic acid derivatives (**Figure 9a**). The two Finnish green cultivars were

separated from black-fruited ones in the score plot of **Figure 9b**. Anthocyanins are the main compounds distinguishing between green and black cultivars.

Figure 9b indicated that myricetins were present at high levels in black cultivars primarily as 3-*O*-glucoside, 3-*O*-rutinoside, deoxyhexoside, 3-*O*-arabinoside and aglycone. 'Venny' and 'Vilma', two green cultivars had high levels of glycosylated kaempferols (Ka-Gal and Ka-Rut) and phenolic acid derivatives (4-Co-Glu, 1-Co-Glu, Co-meGlu-B2, and 1-Ca-Glu).

A large deviation in phenolic content was observed in blackcurrant berries between the two growing years studied, which may be attributed to the influence of weather factors. The PLS plots in **Figure 10** showed that some phenolic compounds correlated strongly to Year 2015, such as quercetin 3-*O*-rutinoside (Qu-Rut), kaempferol 3-*O*-rutinoside (Ka-Rut), 4-*O*-caffeoylglucose (4-Ca-Glu), 4-*O*-coumaroylglucose (4-Co-Glu), 1-*O*-coumaroylglucose (1-Co-Glu), and (*Z*)-coumaroyloxymethylene-glucopyranosyloxy-(*Z*)-butenenitrile (Co-meGlu-B2). No clear correlation was found between growing year and anthocyanins or other secondary metabolites.

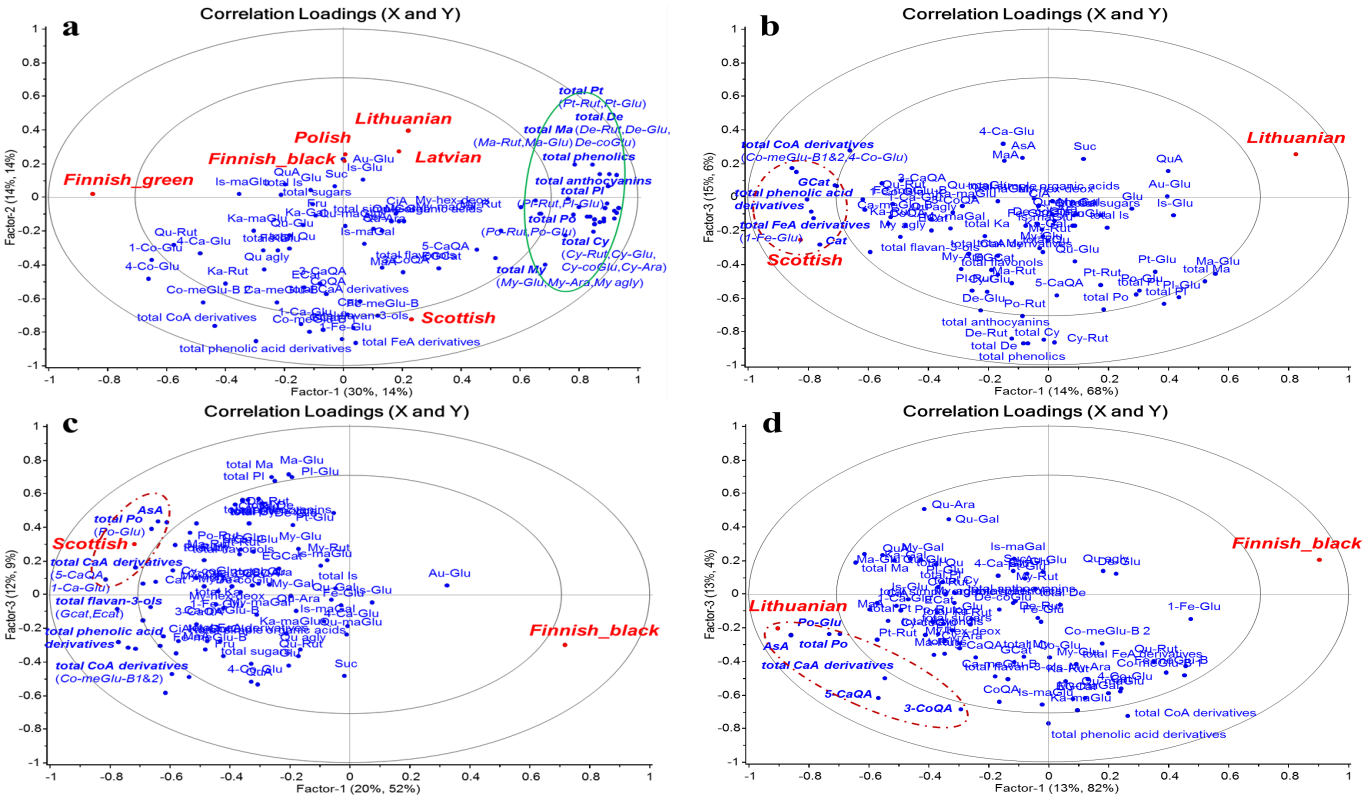
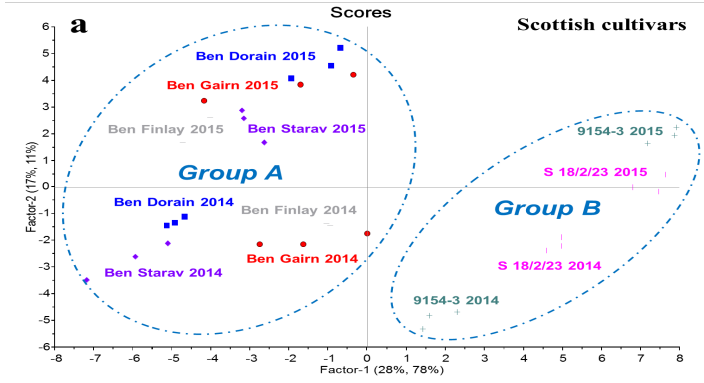
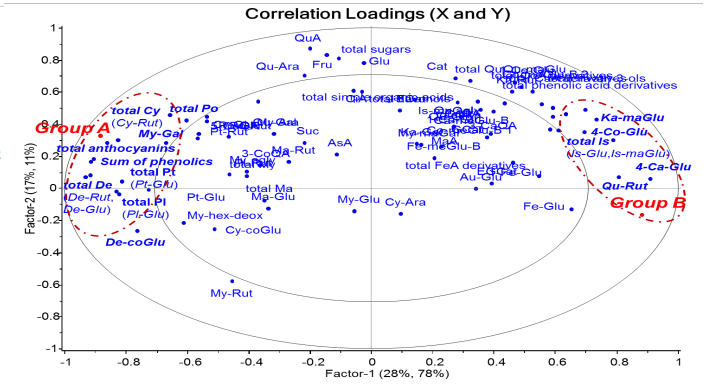
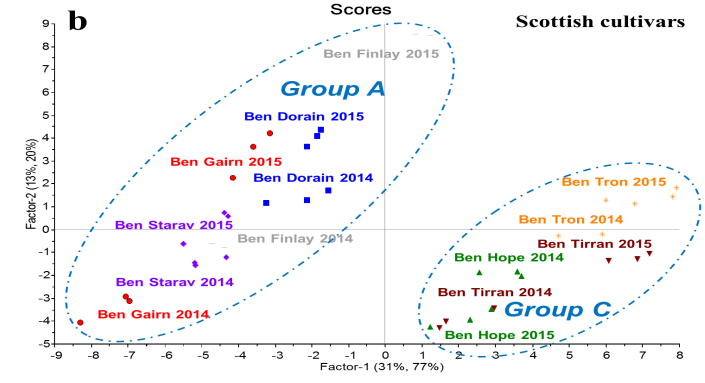
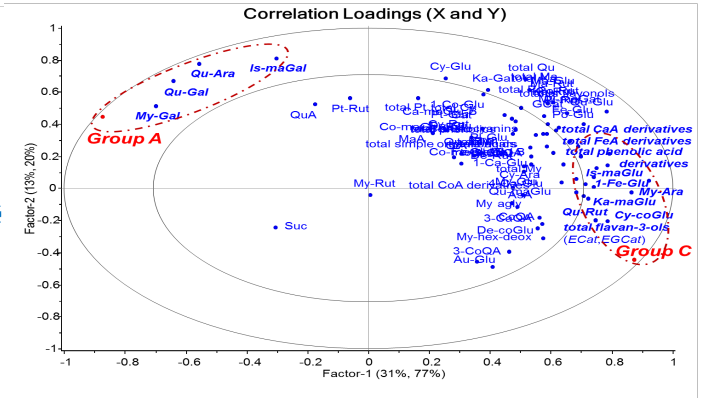


Figure 7. PLS models of blackcurrant cultivars showing the correlation between chemical variables (blue font) and counties of origin (red font): (a) all cultivars; (b) the black samples originating from Scotland and Lithuania; (c) the black samples originating from Scotland and Finland; (d) the black samples of originating from Lithuania and Finland. Reprinted from the original publication **IV** (Tian, Laaksonen, Haikonen, Vanag, Ejaz, Linderborg, Karhu, & Yang, 2019) with permission from American Chemical Society.


a
Scores
Scottish cultivars
Ben Dorain 2015
Ben Gairn 2015
Ben Finlay 2015
Ben Starav 2015
Group A
Ben Dorain 2014
Ben Finlay 2014
Ben Gairn 2014
Ben Starav 2014
9154-3 2015
S 18/2/23 2015
Group B
S 18/2/23 2014
9154-3 2014
Factor-1 (28%, 78%)
Factor-2 (17%, 11%)


Correlation Loadings (X and Y)
QuA
total sugars
Qu-Ara
Fru
Glu
Cat
Group A
total Cy
(Cy-Rut)
total Po
My-Gal
total anthocyanins
Sum of phenolics
total Pt
(Pt-Glu)
total De
(De-Rut,
De-Glu)
total Pl
(Pl-Glu)
De-coGlu
Pt-Glu
My-hex-deox
Cy-coGlu
My-Rut
Suc
3-CoQA
Ma-Rut
AsA
Ma-Glu
total Ma
My-Glu
Cy-Ara
Au-Glu
Fe-Glu
Ka-maGlu
4-Co-Glu
total Is
(Is-Glu,Is-maGlu)
4-Ca-Glu
Qu-Rut
Group B
Factor-1 (28%, 78%)
Factor-2 (17%, 11%)


b
Scores
Scottish cultivars
Ben Finlay 2015
Group A
Ben Dorain 2015
Ben Gairn 2015
Ben Dorain 2014
Ben Starav 2015
Ben Finlay 2014
Ben Starav 2014
Ben Gairn 2014
Ben Tron 2015
Ben Tron 2014
Ben Tirran 2015
Ben Hope 2014
Ben Tirran 2014
Group C
Ben Hope 2015
Factor-1 (31%, 77%)
Factor-2 (13%, 20%)


Correlation Loadings (X and Y)
Qu-Ara
Is-maGal
Qu-Gal
My-Gal
Group A
QuA
Pt-Rut
Cy-Glu
total Qu
Ka-Gal
Fe-Glu
total CoA derivatives
total FeA derivatives
total phenolic acid
derivatives
Is-maGlu
1-Fe-Glu
My-Ara
Ka-maGlu
Qu-Rut
Cy-coGlu
total Flavan-3-ols
(ECat,EGCat)
Group C
My-Rut
Suc
3-CoQA
De-coGlu
My-hex-deox
Au-Glu
Factor-1 (31%, 77%)
Factor-2 (13%, 20%)

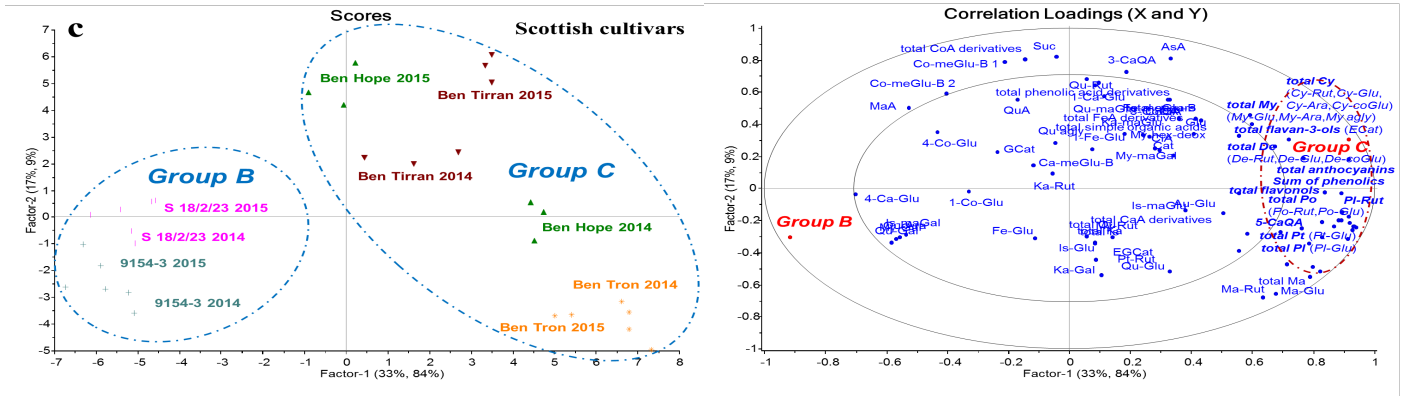


Figure 8. PLS models of blackcurrant cultivars of Scottish origin showing the correlation between chemical variables (blue font) and main groups of cultivars (red font): (a) chemical difference between groups A and B; (b) chemical difference between groups A and C; (c) chemical difference between groups B and C. Reprinted from the original publication **IV** (Tian, Laaksonen, Haikonen, Vanag, Ejaz, Linderborg, Karhu, & Yang, 2019) with permission from American Chemical Society.

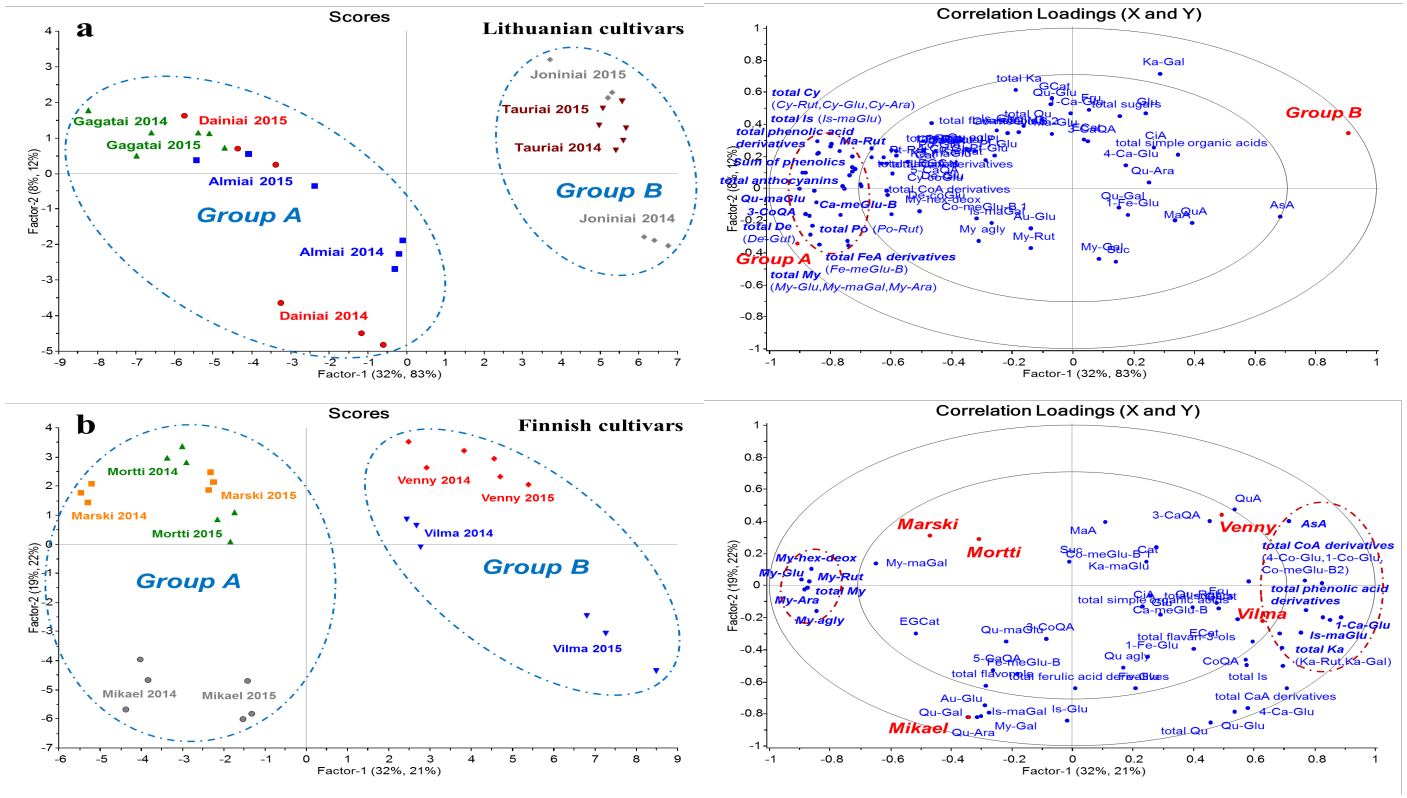


Figure 9. PLS models of blackcurrant cultivars of Lithuanian and Finnish origin showing the correlation between chemical variables (blue font) and main groups of cultivars (red font): (a) Lithuanian cultivars; (b) Finnish cultivars (anthocyanins excluded). Reprinted from the original publication **IV** (Tian, Laaksonen, Haikonen, Vanag, Ejaz, Linderborg, Karhu, & Yang, 2019) with permission from American Chemical Society.

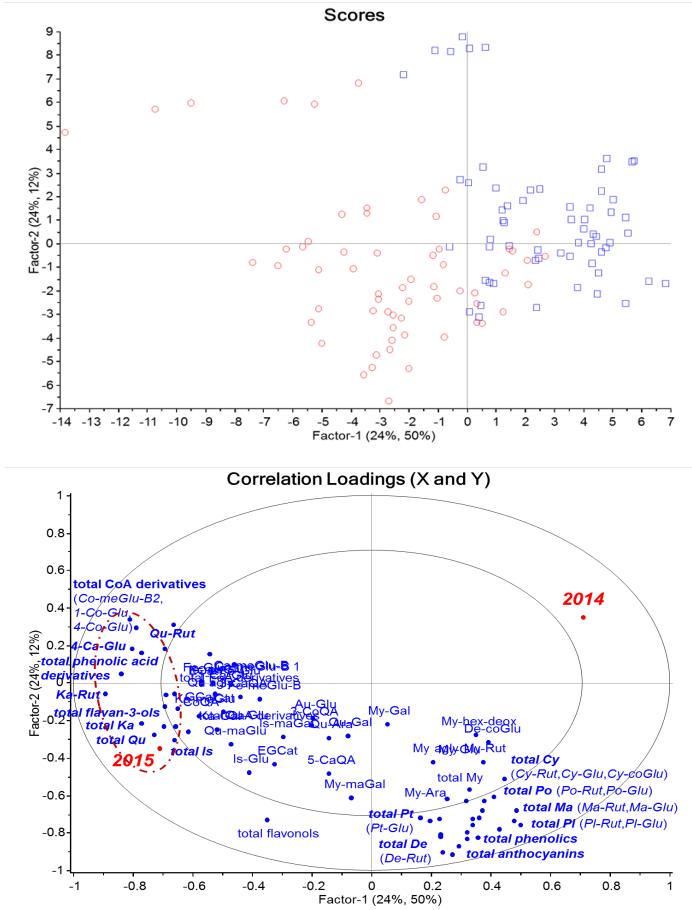


Figure 10. PLS models of blackcurrant cultivars showing the correlation between chemical variables (blue font) and growing year (red font). Reprinted from the original publication **IV** (Tian, Laaksonen, Haikonen, Vanag, Ejaz, Linderborg, Karhu, & Yang, 2019) with permission from American Chemical Society.

6 SUMMARY AND CONCLUSION

Phenolic profiles of leaves, branches, and berries of thirteen Finnish berry species were determined after an extraction protocol using food-grade acidified aqueous ethanol as the extracting solvent. Phenolic compounds were present in these extracts as flavan-3-ols, proanthocyanidins, ellagitannins, phenolic acids, flavonols, flavones, flavanones, anthocyanins, and others. Each extract showed unique phenolic profiles in both composition and concentration. Overall, the leaves were richer sources of phenolics than the berries and branches of the same species and cultivars.

Both the extracts and fractions of the selected extracts were potent inhibitors against free radicals and foodborne pathogens. The total content of phenolics was associated significantly with the free radical scavenging and growth inhibition on microbes. The contribution of phenolics to the activities measured by different anti-oxidative assays differed due to their inherent structures and concentrations presented in the samples. For flavonol glycosides, sugar moieties may play an important role in scavenging peroxyl-radicals. Most of the extracts and fractions inhibited the growth of the target microbes studied. *Bacillus cereus* strains expressed the highest resistance to the berry extracts among all strains studied. The growth inhibition against *Staphylococcus aureus* and *Bacillus cereus* was contributed mostly by ellagitannins and some flavonoids.

The effects of cultivars and growth years on phenolic profiles were also investigated in this research using blackcurrant berries of different cultivars growing in the two consecutive years. The main deviation was in the content of phenolic acid derivatives among cultivars of different origins, and anthocyanins also for those of the same origin. Large variation between growth years was observed in both the concentration and composition of the compounds.

This research provided systematic information on phenolic composition in the food-grade extracts of common Finnish berry plants. *In vitro* bioactivity evaluation (anti-oxidant and anti-bacteria) suggested leaves and berry residue after juice processing to be potential raw materials of natural preservatives for food industry. Since certain leaf and branch extracts contained aromatic compounds which might cause safety issue, fractionation of raw materials is necessary for their potential application.

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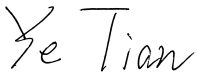
Turku, August 2019



Ye Tian

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DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

1. **REINO R. LINKO (1967)** Fatty acids and other components of Baltic herring flesh lipids. (Organic chemistry).
2. **HEIKKI KALLIO (1975)** Identification of volatile aroma compounds in arctic bramble, *Rubus arcticus* L. and their development during ripening of the berry, with special reference to *Rubus stellatus* SM.
3. **JUKKA KAITARANTA (1981)** Fish roe lipids and lipid hydrolysis in processed roe of certain *Salmonidae* fish as studied by novel chromatographic techniques.
4. **TIMO HIRVI (1983)** Aromas of some strawberry and blueberry species and varieties studied by gas liquid chromatographic and selected ion monitoring techniques.
5. **RAINER HUOPALAHTI (1985)** Composition and content of aroma compounds in the dill herb, *Anethum graveolens* L., affected by different factors.
6. **MARKKU HONKAVAARA (1989)** Effect of porcine stress on the development of PSE meat, its characteristics and influence on the economics of meat products manufacture.
7. **PÄIVI LAAKSO (1992)** Triacylglycerols – approaching the molecular composition of natural mixtures.
8. **MERJA LEINO (1993)** Application of the headspace gas chromatography complemented with sensory evaluation to analysis of various foods.
9. **KAISLI KERROLA (1994)** Essential oils from herbs and spices: isolation by carbon dioxide extraction and characterization by gas chromatography and sensory evaluation.
10. **ANJA LAPVETELÄINEN (1994)** Barley and oat protein products from wet processes: food use potential.
11. **RAIJA TAHVONEN (1995)** Contents of lead and cadmium in foods in Finland.
12. **MAIJA SAXELIN (1995)** Development of dietary probiotics: estimation of optimal *Lactobacillus* GG concentrations.
13. **PIRJO-LIISA PENTTILÄ (1995)** Estimation of food additive and pesticide intakes by means of a stepwise method.
14. **SIRKKA PLAAMI (1996)** Contents of dietary fiber and inositol phosphates in some foods consumed in Finland.
15. **SUSANNA EEROLA (1997)** Biologically active amines: analytics, occurrence and formation in dry sausages.
16. **PEKKA MANNINEN (1997)** Utilization of supercritical carbon dioxide in the analysis of triacylglycerols and isolation of berry oils.
17. **TUULA VESA (1997)** Symptoms of lactose intolerance: influence of milk composition, gastric emptying, and irritable bowel syndrome.
18. **EILA JÄRVENPÄÄ (1998)** Strategies for supercritical fluid extraction of analytes in trace amounts from food matrices.
19. **ELINA TUOMOLA (1999)** *In vitro* adhesion of probiotic lactic acid bacteria.
20. **ANU JOHANSSON (1999)** Availability of seed oils from Finnish berries with special reference to compositional, geographical and nutritional aspects.
21. **ANNE PIHLANTO-LEPPÄLÄ (1999)** Isolation and characteristics of milk-derived bioactive peptides.
22. **MIKA TUOMOLA (2000)** New methods for the measurement of androstenone and skatole – compounds associated with boar taint problem. (Biotechnology).
23. **LEEA PELTO (2000)** Milk hypersensitivity in adults: studies on diagnosis, prevalence and nutritional management.
24. **ANNE NYKÄNEN (2001)** Use of nisin and lactic acid/lactate to improve the microbial and sensory quality of rainbow trout products.
25. **BAORU YANG (2001)** Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries and physiological effects of sea buckthorn oils.
26. **MINNA KAHALA (2001)** Lactobacillar S-layers: Use of *Lactobacillus brevis* S-layer signals for heterologous protein production.
27. **OLLI SJÖVALL (2002)** Chromatographic and mass spectrometric analysis of non-volatile oxidation products of triacylglycerols with emphasis on core aldehydes.
28. **JUHA-PEKKA KURVINEN (2002)** Automatic data processing as an aid to mass spectrometry of dietary triacylglycerols and tissue glycerophospholipids.
29. **MARI HAKALA (2002)** Factors affecting the internal quality of strawberry (*Fragaria x ananassa* Duch.) fruit.
30. **PIRKKA KIRJAVAINEN (2003)** The intestinal microbiota – a target for treatment in infant atopic eczema?
31. **TARJA ARO (2003)** Chemical composition of Baltic herring: effects of processing and storage on fatty acids, mineral elements and volatile compounds.
32. **SAMI NIKOSKELAINEN (2003)** Innate immunity of rainbow trout: effects of opsonins, temperature and probiotics on phagocytic and complement activity as well as on disease resistance.
33. **KAISA YLI-JOKIPII (2004)** Effect of triacylglycerol fatty acid positional distribution on postprandial lipid metabolism.
34. **MARIKA JESTOI (2005)** Emerging *Fusarium*-mycotoxins in Finland.
35. **KATJA TIITINEN (2006)** Factors contributing to sea buckthorn (*Hippophaë rhamnoides* L.) flavour.
36. **SATU VESTERLUND (2006)** Methods to determine the safety and influence of probiotics on the adherence and viability of pathogens.
37. **FANDI FAWAZ ALI IBRAHIM (2006)** Lactic acid bacteria: an approach for heavy metal detoxification.
38. **JUKKA-PEKKA SUOMELA (2006)** Effects of dietary fat oxidation products and flavonols on lipoprotein oxidation.
39. **SAMPO LAHTINEN (2007)** New insights into the viability of probiotic bacteria.
40. **SASKA TUOMASJUKKA (2007)** Strategies for reducing postprandial triacylglycerolemia.

41. **HARRI MÄKIVUOKKO (2007)** Simulating the human colon microbiota: studies on polydextrose, lactose and cocoa mass.
42. **RENATA ADAMI (2007)** Micronization of pharmaceuticals and food ingredients using supercritical fluid techniques.
43. **TEEMU HALTTUNEN (2008)** Removal of cadmium, lead and arsenic from water by lactic acid bacteria.
44. **SUSANNA ROKKA (2008)** Bovine colostral antibodies and selected lactobacilli as means to control gastrointestinal infections.
45. **ANU LÄHTEENMÄKI-UUTELA (2009)** Foodstuffs and medicines as legal categories in the EU and China. Functional foods as a borderline case. (Law).
46. **TARJA SUOMALAINEN (2009)** Characterizing *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 as a new probiotic combination: basic properties of JS and pilot *in vivo* assessment of the combination.
47. **HEIDI LESKINEN (2010)** Positional distribution of fatty acids in plant triacylglycerols: contributing factors and chromatographic/mass spectrometric analysis.
48. **TERHI POHJANHEIMO (2010)** Sensory and non-sensory factors behind the liking and choice of healthy food products.
49. **RIIKKA JÄRVINEN (2010)** Cuticular and suberin polymers of edible plants – analysis by gas chromatographic-mass spectrometric and solid state spectroscopic methods.
50. **HENNA-MARIA LEHTONEN (2010)** Berry polyphenol absorption and the effect of northern berries on metabolism, ectopic fat accumulation, and associated diseases.
51. **PASI KANKAANPÄÄ (2010)** Interactions between polyunsaturated fatty acids and probiotics.
52. **PETRA LARMO (2011)** The health effects of sea buckthorn berries and oil.
53. **HENNA RÖYTIÖ (2011)** Identifying and characterizing new ingredients *in vitro* for prebiotic and synbiotic use.
54. **RITVA REPO-CARRASCO-VALENCIA (2011)** Andean indigenous food crops: nutritional value and bioactive compounds.
55. **OSKAR LAAKSONEN (2011)** Astringent food compounds and their interactions with taste properties.
56. **ŁUKASZ MARCIN GRZEŚKOWIAK (2012)** Gut microbiota in early infancy: effect of environment, diet and probiotics.
57. **PENGZHAN LIU (2012)** Composition of hawthorn (*Crataegus* spp.) fruits and leaves and emblic leafflower (*Phyllanthus emblica*) fruits.
58. **HEIKKI ARO (2012)** Fractionation of hen egg and oat lipids with supercritical fluids. Chemical and functional properties of fractions.
59. **SOILI ALANNE (2012)** An infant with food allergy and eczema in the family – the mental and economic burden of caring.
60. **MARKO TARVAINEN (2013)** Analysis of lipid oxidation during digestion by liquid chromatography-mass spectrometric and nuclear magnetic resonance spectroscopic techniques.
61. **JIE ZHENG (2013)** Sugars, acids and phenolic compounds in currants and sea buckthorn in relation to the effects of environmental factors.
62. **SARI MÄKINEN (2014)** Production, isolation and characterization of bioactive peptides with antihypertensive properties from potato and rapeseed proteins.
63. **MIKA KAIMAINEN (2014)** Stability of natural colorants of plant origin.
64. **LOTTA NYLUND (2015)** Early life intestinal microbiota in health and in atopic eczema.
65. **JAAKKO HIIDENHOVI (2015)** Isolation and characterization of ovomucin – a bioactive agent of egg white.
66. **HANNA-LEENA HIETARANTA-LUOMA (2016)** Promoting healthy lifestyles with personalized, *APOE* genotype based health information: The effects on psychological-, health behavioral and clinical factors.
67. **VELI HIETANIEMI (2016)** The *Fusarium* mycotoxins in Finnish cereal grains: How to control and manage the risk.
68. **MAARIA KORTESNIEMI (2016)** NMR metabolomics of foods – Investigating the influence of origin on sea buckthorn berries, *Brassica* oilseeds and honey.
69. **JUHANI AAKKO (2016)** New insights into human gut microbiota development in early infancy: influence of diet, environment and mother's microbiota.
70. **WEI YANG (2017)** Effects of genetic and environmental factors on proanthocyanidins in sea buckthorn (*Hippophaë rhamnoides*) and flavonol glycosides in leaves of currants (*Ribes* spp.).
71. **LEENAMAIJA MÄKILÄ (2017)** Effect of processing technologies on phenolic compounds in berry products.
72. **JUHA-MATTI PIHLAVA (2017)** Selected bioactive compounds in cereals and cereal products – their role and analysis by chromatographic methods.
73. **TOMMI KUMPULAINEN (2018)** The complexity of freshness and locality in a food consumption context
74. **XUEYING MA (2018)** Non-volatile bioactive and sensory compounds in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*)
75. **ANU NUORA (2018)** Postprandial lipid metabolism resulting from heated beef, homogenized milk and interesterified palm oil.
76. **HEIKKI AISALA (2019)** Sensory properties and underlying chemistry of Finnish edible wild mushrooms.
77. **YE TIAN (2019)** Phenolic compounds from Finnish berry species to enhance food safety.

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