Berry phenolics: isolation, analysis, identification, and antioxidant properties

Petri Kylli

ACADEMIC DISSERTATION

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ABSTRACT

The main objectives in this thesis were to isolate and identify the phenolic compounds in wild (*Sorbus aucuparia*) and cultivated rowanberries, European cranberries (*Vaccinium microcarpon*), lingonberries (*Vaccinium vitis-idaea*), and cloudberries (*Rubus chamaemorus*), as well as to investigate the antioxidant activity of phenolics occurring in berries in food oxidation models. In addition, the storage stability of cloudberry ellagitannin isolate was studied.

In wild and cultivated rowanberries, the main phenolic compounds were chlorogenic acids and neochlorogenic acids with increasing anthocyanin content depending on the crossing partners. The proanthocyanidin contents of cranberries and lingonberries were investigated, revealing that the lingonberry contained more rare A-type dimers than the European cranberry. The liquid chromatography mass spectrometry (LC-MS) analysis of cloudberry ellagitannins showed that trimeric lambertianin C and sanguiin H-10 were the main ellagitannins.

The berries, rich in different types of phenolic compounds including hydroxycinnamic acids, proanthocyanidins, and ellagitannins, showed antioxidant activity toward lipid oxidation in liposome and emulsion oxidation models. All the different rowanberry cultivars prevented lipid oxidation in the same way, in spite of the differences in their phenolic composition. In terms of liposomes, rowanberries were slightly more effective antioxidants than cranberry and lingonberry phenolics. Greater differences were found when comparing proanthocyanidin fractions. Proanthocyanidin dimers and trimers of both cranberries and lingonberries were most potent in inhibiting lipid oxidation.

Antioxidant activities and antiradical capacities were also studied with hydroxycinnamic acid glycosides. The sinapic acid derivatives of the hydroxycinnamic acid glycosides were the most effective at preventing lipid oxidation in emulsions and liposomes and scavenging radicals in DPPH$^{\bullet}$ assay. In liposomes and emulsions, the formation of the secondary oxidation product, hexanal, was inhibited more than that of the primary oxidation product, conjugated diene hydroperoxides, by hydroxycinnamic acid derivatives. This indicates that they are principally chain-breaking antioxidants rather than metal chelators, although they possess chelating activity as well.

The storage stability test of cloudberry ellagitannins was performed by storing ellagitannin isolate and ellagitannins encapsulated with maltodextrin at different relative vapor pressures. The storage stability was enhanced by the encapsulation when higher molecular weight maltodextrin was used. The best preservation was achieved when the capsules were stored at 0 or 33% relative vapor pressures. In addition, the antioxidant activities of encapsulated cloudberry extracts were followed during the storage period. Different storage conditions did not alter the antioxidant activity, even though changes in the ellagitannin contents were seen.

The current results may be of use in improving the oxidative stability of food products by using berries as natural antioxidants.

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LIST OF ORIGINAL PUBLICATIONS

I Kylli P, Nohynek L, Puupponen-Pimiä R, Westerlund-Wikström B, McDougall G, Stewart D, Heinonen M. 2010. Rowanberry phenolics: compositional analysis and bioactivities. J Agric Food Chem 58: 11985-92.

II Kylli P, Nohynek L, Puupponen-Pimiä R, Westerlund-Wikström B, Leppänen T, Welling J, Moilanen E, Heinonen M. 2011. Lingonberry (*Vaccinium vitis-idaea*) and European cranberry (*Vaccinium microcarpon*) proanthocyanidins: isolation, identification, and bioactivities. J Agric Food Chem 59:3373-84.

III Kähkönen M, Kylli P, Ollilainen V, Salminen JP, Heinonen M. 2011. Antioxidant activity of isolated ellagitannins from red raspberries and cloudberries. J Agric Food Chem Submitted for publication.

IV Kylli P, Nousiainen P, Biely P, Sipilä J, Tenkanen M, Heinonen M. 2008. Antioxidant potential of hydroxycinnamic acid glycoside esters. J Agric Food Chem 56: 4797-4805.

V Laine P, Kylli P, Heinonen M, Jouppila K. 2008. Storage stability of microencapsulated cloudberry (*Rubus chamaemorus*) phenolics. J Agric Food Chem 56: 11251-61.

Contribution of the author to papers I-V

I The author planned the study together with the other authors. Compositional analysis, the antioxidant activity testing of rowanberry phenolics, and the writing of the manuscript were carried out by the author. He had the main responsibility for interpreting the results.

II The author planned the study together with the other authors. The isolation and compositional analysis of cranberry and lingonberry proanthocyanidins and the antioxidant testing were carried out by the author. He had the main responsibility for interpreting the results and writing the manuscript.

III The author participated in the LC-MS identification of the cloudberry phenolic isolate.

IV The author planned the study together with the other authors. HPLC analysis, the antioxidant activity testing of hydroxycinnamic acid esters, and the writing of the manuscript were carried out by the author. He had the main responsibility for interpreting the results.

V The author planned the study together with the other authors. The preparation of the phenolic extract, compositional analysis, and the antioxidant activity testing of microencapsulated cloudberry phenolics were carried out by the author. The author had the main responsibility for interpreting the results regarding phenolic composition and antioxidant activity; thus, he was the second author of the paper.

LIST OF ABBREVIATIONS

AS	anthocyanins
ASE	accelerated solvent extractor
DAD	diode array detector
EA	ellagic acid
EI	electron ionization
ESI-MS	electrospray mass spectrometry
ET	ellagitannin
FID	flame ionization detector
FLD	fluorescence detector
GC	gas chromatography
HPLC	high performance liquid chromatography
LC-MS	liquid chromatography mass spectrometry
LDL	low density lipoprotein
LOD	limit of detection
LOQ	limit of quantification
MALDI	matrix assisted laser desorption ionization
MC 18.5	microencapsulated maltodextrins MD18.5
MC 5-8	microencapsulated maltodextrins MD5-8
MD	maltodextrin
MDA	malondialdehyde
OH-B	hydroxybenzoic acid
OH-C	hycroxycinnamic acid
ROS	reactive oxygen species
RVP	relative vapor pressure
SPE	solid phase extraction
TOF	time-of-flight
UPLC	ultra performance liquid chromatography
UV	ultraviolet
Vis	visible

CONTENTS

1 INTRODUCTION

Berries are rich in a large variety of different phenolic compounds, which are considered secondary metabolites. Phenolic compounds such as proanthocyanidins, anthocyanins, flavonols, and phenolic acids predominate in berries (Kähkönen et al. 1999). Proanthocyanidins are the main phenolic group in most berries, such as in the genus *Vaccinium*, including blueberries, cranberries, and lingonberries, and in the genus *Ribes*, encompassing currants and gooseberries (Kähkönen et al. 2001). Dark berries such as bilberries and blackcurrants are found to be rich in anthocyanins (Määttä-Riihinen et al. 2004; Koponen et al. 2007; Ogawa et al. 2008). Flavanols and procyanidins are among the main constituents in some berries, such as in cranberries and lingonberries. Cloudberries and raspberries are members of the genus *Rubus*, which are reported to be rich in ellagitannins (Heinonen et al. 1998; Kähkönen et al. 1999; Kähkönen et al. 2001). Berries belonging to the genus *Fragaria*, including the strawberry, are also rich in ellagitannins, but contain even more anthocyanins. Genera *Rubus* and *Fragaria* belong to the same Rosaceae family. Within berries, the most abundant phenolic acids are caffeic acid and its derivatives (Heinonen et al. 1998), such as esters, glycosides, or cell wall–bound forms. Only a minor fraction of the phenolic acids are in the free form (Chen and Ho 1997). Coffee beverages, apples, rowanberries, and blueberries are observed to contain large amounts of hydroxycinnamic acids. Chlorogenic acid is one of the main hydroxycinnamates found in plants (Mattila et al. 2006). Ferulic acid and *p*-coumaric acid are also abundant, and they are the main hydroxycinnamic acids bound to hemicelluloses and other cell wall materials (Andreasen et al. 2001; Niño-Medina et al. 2010).

Berry phenolics have been shown to have beneficial properties such as antioxidant and antimicrobial activities (Kähkönen et al. 2001; Puupponen-Pimiä et al. 2005). Phenolic acids are considered to be anti-inflammatory, anticarcinogenic, and antimicrobial agents, as well as antioxidants (Silva et al. 2000; Faulds et al. 2004; Puupponen-Pimiä et al. 2005; Nohynek et al. 2006). Various berries, such as hawthorn berries (*Crataegus monogyna*), cloudberries (*Rubus chamaemorus*), raspberries (*Rubus idaeus*), and strawberries (*Fragaria ananassa*), rich in ellagitannins and other phenolic compounds, have been reported to possess antimicrobial properties in relation to the growth of virulent bacteria such as *Helicobacter pylori, Campylobacter jejuni, Candida albicans, Clostridium perfringens, Bacillus subtilis, Listeria monocytogenes,* and *Salmonella* and *Staphylococcus* species (Puupponen-Pimiä et al. 2005; Nohynek et al. 2006; Tadic et al. 2008). Bacterial attachment to host tissues is the first step of infection (Ofek et al. 2003); thus, infections may be avoided by blocking this binding to host cell receptors. Proanthocyanidins and the high-molecular-weight fraction of cranberry (*Vaccinium macrocarpon* Ait.) are known to prevent the adhesion of several bacteria (Foo et al. 2000; Weiss et al. 2002). A-type dimers and trimers have been found predominantly in lingonberries (*Vaccinium vitis-idaea*) and European cranberries (*Vaccinium microcarpon*) or American cranberries (*Vaccinium macrocarpon*). The ingestion of cranberry has traditionally been associated with the prevention of urinary tract infections (UTIs): Kontiokari et al. (2001) and Howell et al. (2005) have found indications that the consumption of cranberry juice can decrease the incidence of recurrent UTI in women. However, there is no clear-cut evidence that the consumption of cranberry juice products prevents UTIs caused by *Escherichia coli* (Howell 2007).

Pharmacological and clinical studies have demonstrated that the flavonoids in berries are the active substances responsible for the anti-inflammatory effect (Raso et al. 2001; Hämäläinen et al 2007). These flavonoids are usually present as glycosides in plants. Several of them have shown inhibitory effects on immunomodulatory mediators in various experimental systems. Quercetin has been shown to have anti-inflammatory properties in several studies. Myricetin, another flavonol, has been shown to exert inhibitory effects in phorbol ester–induced cyclooxygenase (COX)-2 expression in mouse epidermal cells and interleukin (IL)-2 production in mouse EL-4 T-cells. In inflammatory processes, nitric oxide (NO) is produced primarily by the enzyme inducible nitric oxide synthase (iNOS) in inflammatory cells such as macrophages. Aberrant iNOS induction and excessive NO production seem to be involved in the pathophysiology of human inflammatory diseases such as asthma, arthritis, and colitis. Inflammatory response is also enhanced by proinflammatory cytokines IL-1β, IL-6, and tumor necrosis factor (TNF)-α, and by the COX-2 enzyme.

Phenolic compounds found in berries exert numerous effects *in vitro*, but they have to be absorbed from the gut in order to have similar effects in cells. The absorption depends on numerous factors, including molecular structure, the amount consumed, the food matrix, the degree of bioconversion in the gut and tissues, and the nutrient status (Beattie et al. 2005). A variety of anthocyanins appear in urine after supplementation with berries or berry extracts, but in very low concentrations (0.1%) of the dose. Previously, only intact anthocyanins have generally been detected in urine after ingestion. Improved analytical techniques have revealed the presence of low levels of methylated, glucuronidated, and sulfated metabolites (McGhie et al. 2003). Flavonol aglycons such as quercetin are hydrophilic and can passively diffuse across biological membranes. Flavonol glycosides, in contrast, are more water-soluble molecules, which limits their diffusion through cell membranes. Therefore, a transport system is likely involved (Walgren et al. 1998).

Benefits provided by phenolic compounds are assumed to be partly due to their antioxidant activity in chelating metal ions, scavenging radicals, and inhibiting pro-oxidant enzymes (Amorati et al. 2006). The antioxidant activity of polyphenols is attributed to the hydroxyl groups and the availability of phenolic hydrogen for donation (Chen and Ho 1997; Silva et al. 2000). Foods are complex systems comprising various oxidizing components such as lipids and proteins. Due to the complexity of the foods, it is necessary to consider the test conditions of antioxidant activity. The use of simplified, one-dimensional methods that employ nonspecific substrates for evaluating the antioxidant activity in complicated systems makes the interpretation of the results difficult. Therefore, food model systems should be applied when the antioxidant activity of foods is measured. Frankel and Meyer (2000) proposed a testing protocol to evaluate multifunctional food and biological antioxidants. The effectiveness of antioxidants is dependent on the test, and therefore the substrate should be relevant to it. In addition, antioxidant activity testing should be conducted in various oxidation conditions and both primary and secondary oxidation products should be measured.

The phenolic contents of berries vary depending on the family and genus to which they belong. The antioxidant effect and other bioactivities of berry phenolics are strongly dependent on the berry raw material, as the activities differ between the different phenolic constituents. The berries chosen for this study were wild and cultivated rowanberries, cloudberries, European

cranberries, and lingonberries. The phenolic compositions of these berries commonly growing in Finland have not yet been characterized in detail. The antioxidant activity of these berries has not been studied in lipid oxidation models related to food. Therefore, liposome and emulsion oxidation models were chosen to mimic food structures. In this study, the objective was to investigate the different types of berries and their phenolic composition, and to study the influence of the diverse phenolic profiles on the antioxidant activity.

2 LITERATURE REVIEW

2.1 Phenolic compounds in berries

Phenolic compounds are considered secondary metabolites and are derived from phenylalanine. Phenolics can be defined as substances possessing an aromatic ring having one or more hydroxyl groups. Plants contain a large variety of phenolic derivatives, including benzoic acids, cinnamic acid derivatives, flavonoids, isoflavonoids, lignans, and tannins (Shahidi 2000). In berries, the main phenolic classes are hydroxyl benzoic acids, hydroxycinnamic acids, flavonols, anthocyanins, flavan-3-ols and proanthocyanidins, and ellagitannins (**Figure 1**).

Phenolic compounds are formed via the shikimic acid pathway. The amino acid phenylalanine is first formed. Then, the release of ammonia from phenylalanine is catalyzed by phenylalanine ammonia lyase to form *trans*-cinnamic acid, a precursor for several simple phenolic compounds as phenolic acids. *trans*-Cinnamic acid can be converted to para-coumaric acid. The *p*-coumaric acid formed may be hydroxylated further in positions 3 and 5 by hydroxylases and methylated via O-methyl transferase leading, to the formation of caffeic, ferulic, and sinapic acids. These compounds possess a phenyl ring (C6) and a C3 side chain, and are thus collectively termed phenylpropanoids (Naczk and Shahidi 2003; Shahidi and Chandrasekara 2010).

Benzoic acid derivatives are produced when a two-carbon moiety from phenylpropanoids is cleaved. Similar to phenylpropanoid series, the hydroxylation and methylation of hydroxybenzoic acid leads to the formation of dihydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, and gallic acid. Hydroxybenzoic acids are commonly present in the bound form in foods, and are often the component of a complex structure like hydrolyzable tannins. They are also found in the form of organic acids and as sugar derivatives (Naczk and Shahidi 2003; Robert and Mike 2006).

Structures of berry phenolics and their contents in different berries are presented in **Figure 1** and **Table 1**.

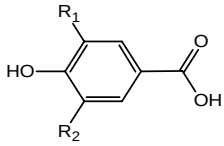
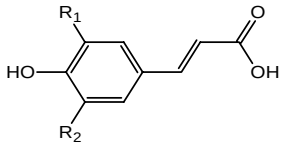
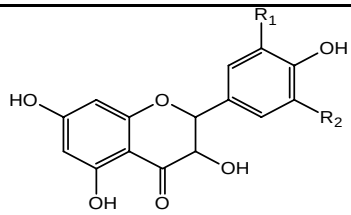
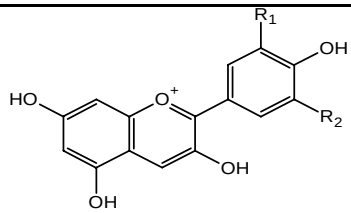
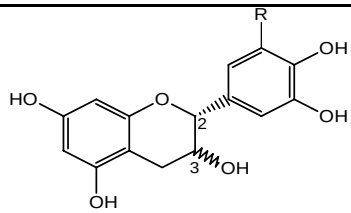
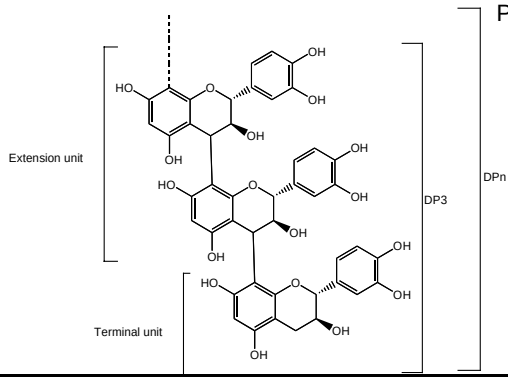
Class	Structure	Trivial names
Hydroxybenzoic acids		*p*-Hydroxybenzoic acid $R_1 = R_2 = H$ Gallic acid $R_1 = R_2 = OH$ Protocatechuic acid $R_1 = OH$, $R_2 = H$ Vanillic acid $R_1 = OCH_3$, $R_2 = H$ Syringic acid $R_1 = R_2 = OCH_3$
Hydroxycinnamic acids		*p*-Coumaric acid $R_1 = R_2 = H$ Caffeic acid $R_1 = OH$, $R_2 = H$ Ferulic acid $R_1 = OCH_3$, $R_2 = H$ Sinapic acid $R_1 = R_2 = OCH_3$
Flavonols		Kaempferol $R_1 = R_2 = H$ Quercetin $R_1 = OH$, $R_2 = H$ Myricetin $R_1 = R_2 = OH$ Isorhamnetin $R_1 = OCH_3$, $R_2 = H$
Anthocyanins		Pelargonidin $R_1 = R_2 = H$ Cyanidin $R_1 = OH$, $R_2 = H$ Delphinidin $R_1 = R_2 = OH$ Peonidin $R_1 = OCH_3$, $R_2 = H$ Petunidin $R_1 = OCH_3$, $R_2 = OH$ Malvidin $R_1 = R_2 = OCH_3$
Flavan-3-ols		(+)-Catechin (2R, 3S) R = H (-)-Epicatechin (2R, 3R) R = H (+)-Gallocatechin (2R, 3S) R = OH (-)-Epigallocatechin (2R, 3R) R = OH
Proanthocyanidins		Procyanidin oligomer

Figure 1. Structures of phenolic compounds in berries.

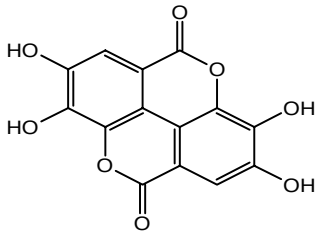
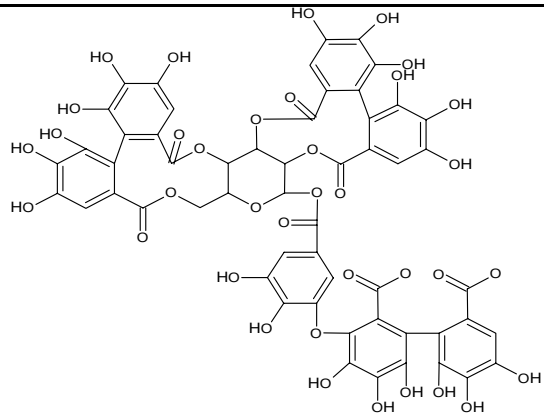
Class	Structure	Trivial names
Ellagic acids		Ellagic acid
Ellagitannins		Ellagitannin dimer

Figure 1. Continued

The flavonoids are formed from the condensation reaction of a phenylpropanoid (C6–C3) compound with malonyl coenzyme A, which leads to the formation of chalcones, which subsequently cyclize under acidic conditions. Thus, flavonoids have the basic skeleton of diphenylpropanes (C6–C3–C6), and depending on the substitution and unsaturation patterns, flavonols, flavan-3-ols, and related compounds are formed. Although the flavonols and flavanols are structurally similar to anthocyanins, they absorb light at shorter wavelengths and thus are not perceived as color. Flavanols and flavonols absorb ultraviolet (UV) light (280 to 365 nm), while anthocyanins absorb UV and visible light (280 and 520 nm) (Naczk and Shahidi 2003; Robert and Mike 2006).

Hydroxybenzoic acids

The hydroxybenzoic acids are *p*-hydroxybenzoic acid, gallic acid, protocatechuic acid, vanillic acid, and syringic acid. The berries of *Vaccinium* species such as bilberries, blueberries, red huckleberries, lingonberries, and *Rubus* species like raspberries, cloudberries, strawberries, and chokeberries are abundant in hydroxybenzoic acids (Määttä-Riihinen et al. 2004a; Määttä-Riihinen et al. 2004b; Taruscio et al. 2004; Mattila et al. 2006; Li et al. 2009). Gallic acid was found to be the most common in raspberries, cloudberries, rosehips, and myrtle berries, whereas protocatechuic acid was predominant in lingonberries, cranberries, gooseberries, and red- and blackcurrants (Mattila et al. 2006; Tuberoso et al. 2010).

Hydroxycinnamic acids

p-Coumaric acid, caffeic acid, and ferulic acid are the most common hydroxycinnamic acids, and are widely distributed in plants (Robbins 2003; Shahidi and Chandrasekara 2010). They occur as bound to the cell wall material or as esters of quinic acid or glucose (Mattila and Kumpulainen 2002). Only a small fraction of hydroxycinnamic acids is in a free form (Robbins 2003). Coffee beverages, apples, rowanberries, and blueberries contain a lot of hydroxycinnamic acids. Chlorogenic acid, which is an ester of caffeic acid and quinic acid (5-*O*-caffeoylquinic acid), is one of the main hydroxycinnamates found in plants (Mattila et al. 2006). Ferulic acid and *p*-coumaric acid are the main hydroxycinnamic acids bound to hemicelluloses and other cell wall materials (Andreasen et al. 2001; Niño-Medina et al. 2010). Sinapic acid has been found in rapeseeds (Vuorela et al. 2003; Thiyam et al. 2006; Khattab et al. 2010). Rowanberries are found to be rich in chlorogenic acid (Kähkönen et al. 2001; Hukkanen et al. 2006; Mattila et al. 2006). Blueberries, bilberries, chokeberries, and saskatoon berries, as well as rowanberries and sweet rowanberry cultivars, have been measured to contain large amounts of caffeic acid derivatives (Määttä-Riihinen et al. 2004a; Määttä-Riihinen et al. 2004b; Taruscio et al. 2004; Hukkanen et al. 2006; Mattila et al. 2006). In blackcurrants, redcurrants, lingonberries, cranberries, cloudberries, raspberries, strawberries, and sea buckthorns, the main hydroxycinnamic acid is *p*-coumaric acid, while the total amount of hydroxycinnamic acids is low (Määttä-Riihinen et al. 2004a; Määttä-Riihinen et al. 2004b; Taruscio et al. 2004; Li et al. 2009; Mattila et al. 2006; Buendía et al. 2010).

Flavonols

Quercetin, myricetin, kaempferol, and isorhamnetin are the main flavonols found in berries. Berries such as blueberries, bilberries, and blackcurrants are substantial in flavonols, especially quercetin (Häkkinen et al. 1999; Määttä-Riihinen et al. 2004a; Borges et al. 2010). Among 18 different berries, bog whortleberry was found to contain the highest concentration of flavonols (Kähkönen et al. 2001; Määttä-Riihinen et al. 2004a).

In bog whortleberries, quercetin derivatives are the prevalent flavonols, followed by myricetin derivatives (Määttä-Riihinen et al. 2004a; Lätti et al. 2009). In one study, American cranberry pomace was detected to contain large amounts of flavonols (White et al. 2010b). *Rubus* berries, cloudberries and raspberries, contain only traces of flavonols (Määttä-Riihinen et al. 2004a). Overall, quercetin derivatives are the most common flavonols and are usually the most abundant, except for in gooseberries, myrtle berries, sea buckthorns, and blackcurrants. The main flavonol in gooseberries is isorhamnetin, while in blackcurrants and myrtle berries, myricetin predominates (Määttä-Riihinen et al. 2004a; Määttä-Riihinen et al. 2004b; Taruscio et al. 2004; Lätti et al. 2009; Borges et al. 2010; Buendía et al. 2010; Tuberoso et al. 2010; Hager et al. 2010).

Anthocyanins

Anthocyanins are pigment compounds in the epidermal tissues in fruits and berries (White et al. 2010a). Anthocyanins are usually present in colored flavylium cation form, but may also be in uncolored form depending on the pH. Anthocyanins have a C6-C3-C6 flavonoid skeleton, and are mostly glycosylated. Glucose, rhamnose, galactose, and arabinose are sugars glycosylated to anthocyanins as 3-glycosides or 3,5-diglycosides. Rutinosides, sophorosides, and sambubiosides also occur. Glycosylation enhances the stability of the anthocyanins (Dao et al. 1998). The anthocyanin aglycons include cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin.

Blackcurrants, bilberries, blueberries, chokeberries, crowberries, and saskatoon berries are rich in anthocyanins (Määttä-Riihinen et al. 2004a; Koponen et al. 2007; Ogawa et al. 2008; Li et al. 2009). Bilberry has been detected to contain 15 different anthocyanins (Kähkönen et al. 2003; Ogawa et al. 2008). Crowberries, along with bilberries, contain delphinidin, cyanidin, petunidin, peonidin, and malvidin glycosides (Määttä-Riihinen et al. 2004a). Delphinidin and cyanidin monoglycosides are the dominant forms, but petunidin, peonidin, and malvidin are also present. Delphinidins are also abundant in blackcurrants (Ogawa et al. 2008). Anthocyanins in lingonberries, redcurrants, red gooseberries, chokeberries, elderberries, rowanberries, cloudberries, and blackberries consist only of cyanidin glycosides (Kähkönen et al. 2003; Määttä-Riihinen et al. 2004a; Määttä-Riihinen et al. 2004b; Ogawa et al. 2008). The main anthocyanins in European cranberries are peonidins, while in American cranberries, cyanidins are the most abundant. Strawberry anthocyanins mainly consist of pelargonidins (Määttä-Riihinen et al. 2004a; Määttä-Riihinen et al. 2004b; Taruscio et al. 2004; Koponen et al. 2007; Ogawa et al. 2008).

Flavan-3-ols and proanthocyanidins

Proanthocyanidins are formed of flavan-3-ol units by interflavan linkages. Flavan-3-ols have the C6-C3-C6 flavonoid skeleton**.** The most common flavan-3-ols are (+)-catechin, (-)-epicatechin, gallocatechin, and epigallocatechin. Procyanidins are formed of (epi)catechin units, while prodelphinidins are made of (epi)gallocatechins. Rare propelargonidins formed from (epi) afzelechins can also be found from berries such as strawberries (McDougall et al. 2008). Procyanidins and mixed procyanidin/prodelphinidin are the most common proanthocyanidins in food. Monomeric flavan units of proanthocyanidins are linked to other flavan units by C-C linkages. The C4 position of the upper unit (extension unit) is connected to the lower unit's (terminal unit) C8 or C6. These are called B-type proanthocyanidins. A-type proanthocyanidins contain two interflavan linkages. The other is the same as in B-type at positions C4-C8 or C4-C6. The second bond is linkage between the position C2 of the upper unit and ether C5 or C7 of the lower unit (Santos-Buelga et al, 2000; White et al. 2010a).

Substantial amounts of proanthocyanidins are found in blackthorns, chokeberries, saskatoon berries, blueberries, cranberries, and lingonberries (Gu et al. 2004; Määttä-Riihinen et al. 2004a; Hellström and Mattila 2008; Buendía et al. 2010; White et al. 2010b; Howard et al. 2010). In blueberries, blackberries, lingonberries, bog whortleberries, elderberries, and chokeberries,

proanthocyanidins are formed only of procyanidins, i.e., (+)-catechin and (-)-epicatechin units, while the proanthocyanidins in bilberries, gooseberries, currants, crowberries, and sea buckthorns consist of procyanidins and prodelphinidins. Strawberry and raspberry proanthocyanidins are formed of procyanidins and propelargonidins (Gu et al. 2004; Määttä-Riihinen et al. 2004a). In most cases, proanthocyanidins occur as B-type. A-type bondages have been found from American cranberries, European cranberries, crowberries, lingonberries, bilberries, and bog whortleberries (Gu et al. 2004; Määttä-Riihinen et al. 2005; Hellström et al. 2009; White et al. 2010b).

Ellagic acids and ellagitannins

Ellagitannins are tannins that consist of esters of hexahydroxydiphenoic acid and polyol (glucose or quinic acid). When ellagitannins are exposed to acids or bases, ester bonds are hydrolyzed, and the released hexyhydroxydiphenic acid is spontaneously rearranged to form ellagic acid, thus they are called hydrolyzable tannins (Buendía et al. 2010). Ellagitannin monomers can further polymerize to form dimers, trimers, and oligomers. In raspberries and blackberries, the most common ellagitannins are sanguiin H-6, lambertianin D, casuarictin, potentillin, pedunculagin (Clifford and Scalbert 2000; Borges et al. 2010). Gasperotti et al. (2010) found that in raspberries and blackberries, lambertianin C and sanguiin H-6 are the main ellagitannins comprising 81% of total ellagitannins in raspberries and 67% in blackberries (Gasperotti et al. 2010).

Ellagitannins have mainly been found in berries belonging to the family of Rosaceae (*Rubus* and *Fragaria* genus). In particular, raspberries, cloudberries, black berries, and arctic brambles (genus *Rubus*) and strawberries (genus *Fragaria*) are rich in ellagitannins (Kähkönen et al. 2001; Määttä-Riihinen et al. 2004b; Koponen et al. 2007; Borges et al. 2010).

Table 1. Contents of phenolic compounds in berries (mg/100g fw).

Species	OH-B	OH-C	Anthocyanins	Flavonols	Flavan-3-ols	Proanthocyanidins	Ellagic acids	ET	Reference
Rosaceae									
arctic bramble *Rubus arcticus*	7.2	5.8	80	13	3.2	2.6	6.4	371	Määttä-Riihinen et al. (2004a)
Azarole *Crataegus azarolus*	0.9	5.0		9.9	39	152			Ganhão et al. (2010)
Blackberry *Rubus fruticosus*	18	45	98 – 152	6.4	1.4 – 2.7	23	2 – 20	23 – 108	Gu et al. (2004); Zadernowski et al. (2005); Ogawa et al. (2008); Jakobek et al. (2009); Hager et al. (2010); Gasperotti et al. (2010)
Blackthorn *Prunus spinosa*	2.1	40 – 45	1.8 – 54	21	1.4 – 28	30 – 302			Määttä-Riihinen et al. (2004a); Ganhão et al. (2010)
Chokeberry *Aronia mitschurinii*	11 – 21	85 – 89	410 – 1313	35	1.9	10 – 659			Gu et al. (2004); Määttä-Riihinen et al. (2004b); Mattila et al. (2006); Koponen et al. (2007); Li et al. (2009)
Cloudberry *Rubus chamaemorus*	11– 30	9.7	1.0 – 1.9	0.5	1.3	0.4	3.3 – 10	312 – 329	Määttä-Riihinen et al. (2004a); Mattila et al. (2006); Koponen et al. (2007)
Common hawthorn *Crataegus monogyna*	0.5	23	0.2	26	415	665			Ganhão et al. (2010)
Dog rose *Rosa canina*	3.9	20	0.7	9.5	500	1961	7.3		Ganhão et al. (2010)
Elm-leaf blackberry *Rubus ulmifolius*	2	11	101	7.6	7.4		27	268	Ganhão et al. (2010)
European juneberry *Amelanchier ovalis*	24	73							Zadernowski et al. (2005)
Raspberry, red *Rubus idaeus*	24 – 129	3.4 – 16	40 – 163	0.3 – 5.7	1.0 – 3.3	0.3 – 26	1.0 – 24	107 – 311	Gu et al. (2004); Määttä-Riihinen et al. (2004a); Mattila et al. (2006); Koponen et al. (2007); Ogawa et al. (2008); Jakobek et al. (2009); Li et al. (2009); Borges et al. (2010); Gasperotti et al. (2010)
Raspberry, yellow *Rubus idaeus*	0.6	2.9		0.6	0.5		3.2	174	Määttä-Riihinen et al. (2004a)
Rose hip *Rosa rugosa*	25	3.9	1.4				2.1	108	Mattila et al. (2006); Koponen et al. (2007)
Rowanberry *Sorbus aucuparia*	0.4 – 1.6	101 – 113	0.5 – 14	26 – 32	26				Mattila et al. (2006); Ganhão et al. (2010)
Saskatoon berry *Amelanchiar alnifolia*	10 – 16	49 – 222	234 – 1079			276	2.0		Mattila et al. (2006); Koponen et al. (2007); Hellström and Mattila (2008); Li et al. (2009)
Strawberry *Fragaria × ananassa*	9.4 – 37	2.6 – 26	32 – 60	2.2 – 6.1	2.4	118 – 141	1.3 – 3.9	17 – 75	Gu et al. (2004); Määttä-Riihinen et al. (2004a); Mattila et al. (2006); Koponen et al. (2007); Ogawa et al. (2008); Li et al. (2009); Buendía et al. (2010)
Sweet rowanberry, burka *S. aucuparia* × *[Sorbus aria* × *Aronia arbutifolia]*		89 – 168	157 – 168	20					Hukkanen et al. (2006); Koponen et al. (2007)
Sweet rowanberry, dessertnaja *S. aucuparia* × *Aronia melanocarpa* L. ×*Mespilus germanica*L.		146	59	37					Hukkanen et al. (2006)
Sweet rowanberry, elliit *S. aucuparia* × *Pyrus* sp.× *S. aucuparia* var. *moravica*		83	25 – 40	16					Hukkanen et al. (2006); Koponen et al. (2007)
Sweet rowanberry, granatnaja *S. aucuparia* × *Crataegus sanguinea*	3.4	68 – 130	87 – 117	12 – 21	8.0	10			Määttä-Riihinen et al. (2004b); Hukkanen et al. (2006); Mattila et al. (2006); Koponen et al. (2007)

OH-B, hydroxybenzoic acids; OH-C, hydroxycinnamic acids; ET, ellagitannins

Table 1. Continued

Species	OH-B	OH-C	Anthocyanins	Flavonols	Flavan-3-ol	Proanthocyanidins	Ellagic acids	ET	Reference
Sweet rowanberry, kubovaja *S. aucuparia* sp.		180	7	24					Hukkanen et al. (2006)
Sweet rowanberry, rosina *S. aucuparia* var. *moravica*		132	5.7	17					Hukkanen et al. (2006)
Sweet rowanberry, rubinovaja *S. aucuparia* × *Pyrus communis* L.		185	89	32					Hukkanen et al. (2006)
Sweet rowanberry, titan Burka × *Malus* sp. × *Pyrus* sp.		120	102	18					Hukkanen et al. (2006)
Sweet rowanberry, zoltaja *S. aucuparia* × *Pyrus* sp.		203	10	24					Hukkanen et al. (2006)
Ericaceae									
Bilberry *Vaccinium myrtillus*	27	18 – 27	564 – 873	11 – 14	7.5	0.1–1.0			Määttä-Riihinen et al. (2004b); Hukkanen et al. (2006); Mattila et al. (2006); Koponen et al. (2007); Ogawa et al. (2008)
Blueberry, half-highbush *Vaccinium angustifolium Aiton*× *corymbosum* L.		34 – 163	67 – 148	11 – 12	1.3 – 4.2	0.1–1.0			Määttä-Riihinen et al. (2004b); Taruscio et al. (2004)
Blueberry, highbush *Vaccinium corymbosum* L		0.3 – 150	42 – 216	9.9 – 43	4.0 – 5.3	176			Gu et al. (2004); Taruscio et al. (2004); Borges et al. (2010)
Blueberry, lowbush *Vaccinium angustifolium*	19	57			3.4	328			Gu et al. (2004); Zadernowski et al. (2005)
Blueberry, oval-leaf *Vaccinium ovalifolium*	0.2	4.7	218	7.3	11				Taruscio et al. (2004)
Blueberry, wild *Vaccinium corymbosum* spp.	24 – 61	63 – 155	149 – 997			31	2.4		Mattila et al. (2006); Koponen et al. (2007); Ogawa et al. (2008); Li et al. (2009); Howard et al. (2010)
Bog whortleberry *Vaccinium uliginosum*	0.9 – 19	1.5 – 29	74 – 351	6.4 – 154	2.2 – 7.6				Määttä-Riihinen et al. (2004b); Taruscio et al. (2004); Mattila et al. (2006); Koponen et al. (2007); Lätti et al. (2009)
Cranberry, American *Vaccinium macrocarpon*	0.8 – 6.9	4.2 – 22	30 – 74	23 – 26	18	412			Gu et al. (2004); Taruscio et al. (2004); Mattila et al. (2006); Borges et al. (2010)
Cranberry, European *Vaccinium oxycoccus*		7.6	66 – 86	27	3.1	1.5–2.0			Määttä-Riihinen et al. (2004b); Koponen et al. (2007); Ogawa et al. (2008)
Cranberry, pomace *Vaccinium macrocarpon*			121	358		167			White et al. (2010a)
Huckleberry, black leaf *Vaccinium membranaceum*	0.2	12	130	7.3	24				Taruscio et al. (2004)
Huckleberry, cascade *Vaccinium deliciosum*	0.7	15	100	4.8	11				Taruscio et al. (2004)
Huckleberry, evergreen *Vaccinium ovatum*	1.2	61	185	9.4	6.9				Taruscio et al. (2004)
Huckleberry, red *Vaccinium parvifolium*	55	35	11	0.9	15				Taruscio et al. (2004)
Lingonberry *Vaccinium vitis-idaea*	15	10 – 11	78 – 130	13 – 17	26	30 – 158			Määttä-Riihinen et al. (2004b); Hukkanen et al. (2006); Mattila et al. (2006); Koponen et al. (2007); Hellström and Mattila (2008)
Strawberry tree *Arbutus unedo*	32	0.3	1.7	1.0	89	136	2.0		Ganhão et al. (2010)

OH-B, hydroxybenzoic acids; OH-C, hydroxycinnamic acids; ET, ellagitannins

Table 1. Continued

Species	OH-B	OH-C	Anthocyanins	Flavonols	Flavan-3-ol	Proanthocyanidins	Ellagic acids	ET	Reference
Grossulariaceae									
Blackcurrant *Ribes nigrum*	12 – 19	2.2 – 53	201 – 488	16 – 25	1.9	10 – 147			Gu et al. (2004); Määttä-Riihinen et al. (2004b); Zadernowski et al. (2005); Mattila et al. (2006); Koponen et al. (2007); Ogawa et al. (2008); Borges et al. (2010)
Redcurrant *Ribes rubrum*	4.3	1.6 – 4.0	15 – 42	0.4 – 3.5	0.7	0.1–1.0			Määttä-Riihinen et al. (2004b); Mattila et al. (2006); Koponen et al. (2007); Ogawa et al. (2008); Borges et al. (2010)
Whitecurrant *Ribes glandulosum*		4.1		1.3	1.9	0.1–1.0			Määttä-Riihinen et al. (2004b)
Gooseberry, red *Ribes uva-crispa*	2.4	4.3 – 5.5	24 – 32	3.2	2.8	0.1–1.0			Määttä-Riihinen et al. (2004b); Mattila et al. (2006); Koponen et al. (2007);
Gooseberry, yellow *Ribes uva-crispa*	0.3	4.7 – 5.1		2.9	0.8	0.1–1.0			Määttä-Riihinen et al. (2004b); Mattila et al. (2006)
Caprifoliaceae									
Blue-berried honeysuckle *Lonicera caerulea* var. *camtschatica Sevast*	23	52							Zadernowski et al. (2005)
Elderberry *Sambucus nigra* L.		20	332	33		0.1–1.0			Määttä-Riihinen et al. (2004b)
Elaeagnaceae									
Sea buckthorn *Hippophae rhamnoides* L.	0.1 – 8.4	6.8 – 10		42	0.6	0.1–1.0		1.0	Määttä-Riihinen et al. (2004b); Mattila et al. (2006); Koponen et al. (2007); Li et al. (2009)
Empetraceae									
Northern crowberry *Empetrum hermaphroditum*		15	768	1.7	12	10			Määttä-Riihinen et al. (2004b)
Southern crowberry *Empetrum nigrum*	23	6.0 – 9.4	360 – 531	5.9	6.0	0.1–1.0			Määttä-Riihinen et al. (2004b); Mattila et al. (2006); Koponen et al. (2007); Ogawa et al. (2008)
Moraceae									
Black mulberry *Morus nigra* L.	6.9	47	291						Zadernowski et al. (2005); Ogawa et al. (2008)
Myrtaceae									
Myrtle berry *Myrtus communis*	19 – 50		3.6 – 220	10 – 149			0.8 – 11		Tuberoso et al. (2010)

OH-B, hydroxybenzoic acids; OH-C, hydroxycinnamic acids; ET, ellagitannins

2.2 Extraction and purification of berry phenolics

Phenolic compounds in plants and berries are polar compounds, which usually are extracted with polar solvents such as aqueous acetone and methanol. Aqueous acetone has been shown to be a more efficient extraction solvent than aqueous methanol for hydroxycinnamic acids and anthocyanins (Heinonen et al. 1998; Kähkönen et al. 2001). On the other hand, aqueous methanol may be better for flavan-3-ols and proanthocyanidins (Kähkönen et al. 2001). A small addition of organic acids (formic acid, acetic acid) may stabilize anthocyanins and enhance their extractability (Gao and Mazza 1994; Kalt et al. 2008). The portion of organic acids in extraction solvents varies from 0.01% to 10%. Other extraction solvents have also been used. Tuberoso et al. (2010) extracted phenolic compounds from myrtle berries with ethanol, ethyl acetate, and water. Differences between solvents were found: ethanol was the best solvent, ethyl acetate was moderate, and water was the least effective. The phenolic profiles also differed when different solvents were used. Ethanol extracted more polar compounds such as anthocyanins, while ethyl acetate extracted more nonpolar flavonols. Singh et al. (2011) tested the solvent efficacy of 80% aqueous acetone, methanol, and ethanol for flavonols from curry leaves. In the conclusion of their study, aqueous ethanol extracted flavonols most effectively, followed by aqueous methanol and acetone. Extractions are almost always repeated 2–3 times with extracts, and then combined. To extract the phenolic compounds from fresh or lyophilized material, samples are mixed with the extraction solvent. Vortexing, shaking, or blending with Ultra Turrax can enhance the yields of the extracts. Freeze drying is usually employed prior to extraction.

C18 Solid phase extraction (SPE) is useful for removing sugars, and to some extent, organic acids from phenolic extracts. Phenolic compounds are eluted with acidified polar solvent. The disadvantage in using SPE is that flavan-3-ols and proanthocyanidins may bind to the sorbent material (Kähkönen et al. 2001). The yields of proanthocyanins increased when the acid portion was changed to water (Kalt et al. 2008). Recoveries of the phenolic extracts after SPE are not commonly reported, but according to a few publications, the recoveries were almost 100% (Glowniak et al. 1996; Benassi and Cecchi 1998; Pinelo et al. 2006).

Hydrolysis has been used to simplify the chromatographic analysis of phenolic compounds. Acid or alkaline hydrolysis is applied prior to the analysis. Acid hydrolysis cleaves glycosidic bonds between the phenolic compound and the sugar molecule attached to it. Alkaline hydrolysis has been employed in order to hydrolyze ester bonds. Phenolic acids such as ferulic acid and *p*-coumaric acids are mainly bound to cell walls, and have to be released by alkaline hydrolysis (Madhujith et al. 2009; Verma et al. 2009). Cinnamoyl esterases have commonly been used for the enzymatic release of phenolic acids from cell wall polysaccharides in grains (Bartolomé and Gómez-Cordovés 1999; Faulds et al. 2002; Yu et al. 2002). Pectinolytic enzymes that cleave the carbohydrate backbone disrupt the cell wall structure and enhance the extractability of phenolic compounds. Pectinases have been utilized for the extraction of bilberry and blackcurrant phenolics (Koponen et al. 2008; Puupponen-Pimiä et al. 2008). Unfortunately, the hydrolysis loses the valuable information of the naturally occurring glycosylates, as well as of other conjugates and their bioactivities.

Phenolic acids

The extraction of phenolic acids has been carried out with aqueous acetone (Kähkönen et al. 2001), aqueous methanol (Häkkinen et al. 1999; Kähkönen et al. 2001; Mattila et al. 2006; Zadernowski et al. 2009), or ethyl acetate (Määttä-Riihinen et al. 2004a) (**Table 2**). Often, phenolic acids are ester linked to cell walls, and thus have to be released by saponification. For this, NaOH is employed (Kroon et al. 1997). In most cases, in order to simplify the data handling and interpretation of the results, extracts have been hydrolyzed for 16 h at 35–40 °C with acids, usually HCl, prior to analysis. For example, chlorogenic acids are often analyzed as caffeic acid after acid hydrolysis (Häkkinen and Auriola 1998; Mattila and Kumpulainen 2002; Määttä-Riihinen et al. 2004a; Mattila et al. 2006). To protect phenolic acids from degradation during the acid hydrolysis, antioxidants such as *tert*-butylhydroquinone or ascorbic acid have been added.

Flavonols

The extraction of flavonols from different plant materials has been carried out with alcoholic or organic solvents (**Table 2**). Ethanol or methanol has proven to be the most efficient solvent for flavonol extraction, especially as an aqueous solution (Lätti et al. 2009; Mohdaly et al. 2010; Singh et al. 2011). To analyze flavonols as aglycons, they need to be hydrolyzed. Häkkinen and Törrönen (2000) performed hydrolysis in 50% aqueous methanol containing 1.2 M HCl and *tert*-butylhydroquinone as an antioxidant by refluxing at 85 °C for 2 h.

Anthocyanins

Anthocyanins have been extracted with acidified 80% aqueous methanol (Kähkönen et al. 2003; Cuevas-Rodríguez et al. 2010). A small amount of acid (TFA, acetic acid, formic acid) is usually added in order to stabilize anthocyanins. For further purification, the extract is applied to an Amberlite XAD-7 column, and sugars and phenolic and organic acids are washed out with acidified water. The remaining phenolic fraction is eluted with methanol. After XAD-7 fractionation, the extract contains anthocyanins and proanthocyanidins. To separate the anthocyanins from proanthocyanidins, Sephadex LH-20 column chromatography can be applied. Anthocyanins are eluted with aqueous methanol, followed by proanthocyanidins with aqueous acetone.

Flavan-3-ols and proanthocyanidins

Flavan-3-ols and low-molecular-weight proanthocyanidins can be extracted with ethyl acetate (Määttä-Riihinen et al. 2004b). It has been shown that high-molecular-weight proanthocyanidins are extracted more efficiently with a combination of acetone, methanol, and water (2:2:1) containing 0.01% formic acid (Hellström and Mattila 2008; Kalt et al. 2008). For further purification, column chromatography such as Sephadex LH-20 can be used.

Ellagic acids and ellagitannins

Ellagitannins have been extracted with 70% aqueous acetone followed by Sephadex LH-20 column chromatography fractionation by subsequent elution with water, aqueous methanol, or ethanol and aqueous acetone (Hager et al. 2008; McDougall et al. 2008; Gasperotti et al. 2010; Karonen et al. 2010). Another approach for ellagitannin extraction is to use ethanol:water:formic acid (80:20:1) as a solvent and separate ellagitannins from anthocyanins by the ion exchange column. In this procedure, ellagitannins were eluted with the extraction solvent and anthocyanins ethanol:water:HCl (50:50:1) (Kool et al. 2010) (**Table 2**).

Table 2. Extraction methods of berry phenolics.

Material	Target	Solvent [a]	Reference
Bilberry	phenolics	pectinase enzymes	Puupponen-Pimiä et al. (2008)
Blueberry	phenolics	acetone/water/HAc (60:30:10)	Brambilla et al. (2008)
Cranberry, blueberry, raspberry	phenolics	methanol, supercritical CO_2	Laroze et al. (2010)
Myrtle berry	phenolics	Ethanol water ethyl acetate	Tuberoso et al. (2010)
Blackcurrant, blueberry, raspberry, redcurrant, cranberry	phenolics	MeOH/HAc (99:1)	Borges et al. (2010)
European cranberrybush	phenolics	MeOH/water/EtAc (80:19.9:0.1)	Sedat Velioglu et al. (2006)
Bilberry, lowbush blueberry, highbush blueberry, rabbiteye blueberry, cranberry, partridgeberry, lingonberry, strawberry	phenolics	acetone/MeOH/water/HAc (40:40:20: 0.01), SPE C18	Kalt et al. (2008)
Bilberry, lowbush blueberry, highbush blueberry, rabbiteye blueberry, cranberry, partridgeberry, lingonberry, strawberry	phenolic	Acetone/MeOH/HAc (23:77: 0.01)	Kalt et al. (2007)
Blueberry, huckleberry, cranberry, bilberry	phenolics	acetone/water (70:30)	Taruscio et al. (2004)
26 different berries	phenolics	MeOH/water (60:40), acetone/water (70:30), hexane, water	Kähkönen et al. (2001)
Blackberry, highbush blueberry, red raspberry, strawberry, cherry	phenolics	MeOH/water (60:40) acetone/water (70:30)	Heinonen et al. (1998)
Cranberry pomace	phenolics	acetone/water/EtAc (70:29.5:0.5)	White et al. (2010a)
Blueberries	phenolics	MeOH/water/HAc (60:37:3)	Howard et al. (2010)
Blackcurrant, strawberry	flavonols, phenolic acids	0.6 M HCl hydrolysis, methanol	Häkkinen et al. (1998)

[a] MeOH, methanol; ACN, acetonitrile; HAc, formic acid; EtAc, acetic acid

Table 2. Continued

Material	Target	Solvent [a]	Reference
Strawberry, blueberry, bilberry, bog whortleberry	flavonoids, phenolic acids	MeOH/water (50:50) containing 1.2M HCl	Häkkinen and Törrönen (2000)
Bilberry, blackcurrant	flavonols	pectinase enzymes, 80% MeOH + 2% HCl in water, 50% MeOH + 1% HCl in water	Koponen et al. (2008)
23 different berries	anthocyanins	MeOH/water/HCl (80:18:2) MeOH/water/HCl (50:49:1) MeOH/water/HCl (66.7:16.6:16.6)	Koponen et al. (2007)
Bilberry, blackcurrant	anthocyanins	pectinase enzymes, 80% MeOH + 2% HCl in water, 50% MeOH + 1% HCl in water	Koponen et al. (2008)
Bilberry, blackberry, blackcurrant, blueberry, cranberry, crowberry, mulberry, raspberry, redcurrant, strawberry	anthocyanins	MeOH/water/EtAc (80:19.5:0.5)	Ogawa et al. (2008)
Bilberry, lowbush blueberry, highbush blueberry, rabbiteye blueberry, cranberry, partridgeberry, lingonberry, strawberry	proanthocyanidins	Acetone/MeOH/HAc (23:77: 0.01) SPE C18 Sephadex LH-20	Kalt et al. (2007)
Bilberry, lowbush blueberry, highbush blueberry, rabbiteye blueberry, cranberry, partridgeberry, lingonberry, strawberry	proanthocyanidins	acetone/MeOH/water/HAc (40:40:20: 0.01), SPE C18 acetone/water (70:30), ethyl acetate, Sephadex LH-20	Kalt et al. (2008)
Blueberry, huckleberry, cranberry, bilberry	proanthocyanidins	MeOH, 6 M HCl hydrolysis	Taruscio et al. (2004)
Cranberry pomace	proanthocyanidins	acetone/water/EtAc (70:29.5:0.5) Sephadex LH-20	White et al. (2010a)
Cranberry pomace	proanthocyanidins	2 M, 4 M and 6 M NaOH + ethyl acetate	White et al. (2010c)
Strawberry	proanthocyanidins	acetone/water/EtAc (70:29.5:0.5) SPE C18	Buendía et al. (2010)
Blueberries	proanthocyanidins	MeOH/water/HAc (60:37:3) acetone/water/EtAc (70:29.5:0.5) Sephadex LH-20	Howard et al. (2010)
Blackberry	ellagitannins	acetone/water/EtAc (70:29.5:0.5)	Hager et al. (2010)

[a] MeOH, methanol; ACN, acetonitrile; HAc, formic acid; EtAc, acetic acid; NaOH sodium hydroxide

2.3 Methods for analysis and identification of berry phenolics

2.3.1 Spectrophotometric methods

The total phenolic content of berry extracts is usually determined spectrophotometrically by the Folin-Ciocalteau method. This method is based on the reduction of phosphomolybdic-phosphotungstic acid reagent (Folin reagent) in alkaline solution by phenolic compounds (Singleton and Rossi 1965). Samples in methanol or water are mixed with Folin reagent and sodium carbonate, and the absorbance is measured at 755–765 nm after 30 min (Naczk and Shahidi 1989; Benvenuti et al. 2004; Skrede et al. 2004; Prior et al. 2005; Pinelo et al. 2006; Madrigal-Carballo et al. 2009). Usually, the results are expressed as gallic acid equivalents, but other phenolic acids have also been used for the quantification. Berry extracts may contain other reducing agents (sugars and proteins, for example), which may result in the overestimation of phenolic content.

Anthocyanins are known to display color variations at different pH values. In acidic aqueous media (pH 1.0) anthocyanins exist mainly as red flavylium cation. When the pH is increased (pH 4.5), the colorless carbinol form prevails. The pH-differential method is based on this reaction. Samples are measured at two wavelengths: 510–550 nm (absorbance maximum for anthocyanins) and 700 nm (for haze correction), and at two different pH levels: 1.0 and 4.5. The absorbance is calculated as follows:

$$A = (A\lambda_{\text{vis-max}} - A_{700})_{\text{pH 1.0}} - (A\lambda_{\text{vis-max}} - A_{700})_{\text{pH 4.5}}$$

The concentration of anthocyanins is then calculated by the Lambert-Beer equation using the molar absorptivity (ε) of cyanidin-3-glucoside if the ε for the predominant anthocyanin is unknown (Giusti and Wrolstad 2001).

Vanillin assay has widely been employed for the determination of proanthocyanidins in plants. The aldehyde vanillin reacts with meta-oriented hydroxyl groups on the flavanol A-ring. Usually, 0.5–2% vanillin in an acidic methanol is used for the reaction (Sun et al. 1998b). After 15 min incubation at 30 °C, the absorbance is measured at 500510 nm, depending on the acid used. When HCl or H_2SO_4 is used, the absorbance is read at 500 nm, but when glacial acetic acid is employed, the absorbance is measured at 510 nm (Price et al. 1978; Butler et al. 1982; Sun et al. 1998b; Wallace and Giusti 2010). In many fruit and berry samples, anthocyanins absorb light at the same wavelength region; thus, the anthocyanins present may cause overestimation of the proanthocyanidins.

The 4-dimethylaminocinnamaldehyde (DMAC) assay is similar to the vanillin assay; aldehyde reagent reacts at the same position of the proantocyanidin as in the vanillin assay. The product of the DMAC reaction has its maximum wavelength at 640 nm, thus eliminating the interference of anthocyanins (Wallace and Giusti 2010). According to Wallace and Giusti (2010), for the proanthocyanidin analysis, it is best to incubate samples with 2% DMAC in 3 M H_2SO_4 at

room temperature for 15 to 35 min. High-throughput 96-well plate methods have also been developed, where 0.1% DMAC solution in HCl acidified ethanol was used for the reaction. The plates were measured at 640 nm after 25 min (Payne et al. 2010; Prior et al. 2010). Monomeric flavan-3-ols have been observed to be more reactive towards the DMAC reagent than oligomeric and polymeric proanthocyanidins. Different proanthocyanidins also possess different extinction coefficients, making the comparison between the berry species difficult if the proanthocyanidin profiles differ.

2.3.2 Liquid chromatography (HPLC)

Liquid chromatography is one of the most common techniques to analyze phenolic compounds in berries, and reversed phase high-performance liquid chromatography (RP-HPLC) is the most common technique among them. In RP-HPLC, non-polar C-18 or C-8 columns are used with the gradient of polar solvents. **Table 3** summarizes the HPLC methods for phenolic compound analysis. Most of the methods are performed with reversed phase column water-based solvents as eluent and acetonitrile or methanol as organic modifier. Usually, eluents contain 0.1–5% aqueous acetic, formic, or trifluoroacetic acid to enhance the retention to non-polar column. Normal phase columns such as silica or diol are mainly used for proanthocyanidin separation. Unlike in RP-HPLC, in which the separation is based mainly on the polarity, proanthocyanidins are eluted in normal phase high-performance liquid chromatography (NP-HPLC) systems according to their degree of polymerization. The gradient of dichloromethane and methanol in aqueous formic or acetic acid is usually applied in NP-HPLC (Hümmer and Schreier 2008).

The identification of phenolic compounds has usually been based on the spectral properties using ultraviolet-visible (UV-Vis), fluorescence (FLD), mass spectroscopy (MS), or nuclear magnetic resonance (NMR). Phenolic compounds exhibit their absorbance maximum at the 275–285 nm wavelength region due to aromatic ring in their molecular structure. Phenolic acids and flavonoids have characteristic UV-Vis absorbances: hydroxybenzoic acids are detected at 280 nm, hydroxycinnamic acids at 320 nm, flavonols and ellagic acid at 365 nm, and anthocyanidins at 520 nm. Proanthocyanidins absorb at 280 nm but the fluorescence detection is more suitable, as it is selective for proanthocyanidins (Hümmer and Schreier 2008). The excitation wavelength is usually set to 275–280 nm and the emission wavelength to 315–325 nm. The sensitivity of fluorescence detection is much higher than that of UV-Vis (Gu et al. 2002).

Mass spectrometry has become a general method for detecting and tentatively identifying phenolic compounds. Electrospray ionization (ESI) and atmospheric chemical ionization (APCI) are the main ionization techniques for HPLC. ESI is applicable for polar compounds, while APCI is better for neutral and nonpolar compounds. These ionization techniques are considered soft ionization, producing the quasi-molecular ions $[M+H]^+$ or $[M-H]^-$ (Ketola et al. 2010). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) has emerged as a technique for polymeric, nonvolatile, and polar phenolic compounds such as proanthocyanidins and ellagitannins. For this detection, quadrupole and ion trap mass analyzers are commonly used. Conventional LC-MS coupled to quadrupole or ion trap may not be suitable for analyzing higher molecular weight compounds due to the limit of the mass

range of quadrupole and ion trap mass analyzers, which is around 2000–4000 Da (Ketola et al. 2010). Higher molecular weight compounds may be detected as multiply charged, and therefore analyzed with LC-MS (Mullen et al. 2003; Gasperotti et al. 2010). MALDI-TOF or HPLC coupled to time-of-flight mass spectrometry can be used instead because of the wider mass range of these approaches (up to 300 kDa) (Ketola et al. 2010; Kool et al. 2010).

2.3.3 Gas chromatography (GC)

Although HPLC and LC-MS methods are the most used for the analysis of phenolic compounds, the gas chromatography with flame ionization detector (GC-FID) and gas chromatography mass spectrometry (GC-MS) methods have also been developed. These methods have been employed mainly for phenolic acid analysis (Soleas et al. 1997; Chu et al. 2001; Plessi et al. 2006), but also for flavonols (Zhang and Zuo 2004). Prior to the analysis, phenolic compounds have to be derivatized; trimethylsilyl (TMS) derivatives are most widely used. Phenolic acids and flavonols are then detected as $[M+TMS]^+$ in MS. Generally, GC-MS systems with electron ionization (EI) are used; thus, MS libraries may be utilized for the identification of the compounds. EI is one of the oldest and simplest ionization methods, where the analyte molecules are beamed with electrons to form molecular ion radical cation ($M^{+\bullet}$). Usually, fragment ions are also formed beside the molecular ion. The fragmentation for most organic compounds is relatively repeatable and makes possible the use of mass spectra libraries (Hübschmann 2009). García-Villalba et al. (2011) developed and validated a GC-APCI-TOF method for the analysis of phenolic compounds in olive oil. In this approach, sample preparation is more time consuming compared to LC methods; however, GC systems are more stable, repeatable, and selective. GC-MS methods are suitable only for relatively small molecules (molecular weight below 600 Da) due to the need for volatility (Zadernowski et al. 2005; Kivilompolo et al. 2007; Zadernowski et al. 2009).

Table 3. HPLC methods of phenolic compounds.

Solvents[a]			Detectors	Runtime	Phase[b]	Type of phenolics	Reference
A	B	C					
0.5% TFA in water	MeOH	-	ESI-MS	24 min	RP	anthocyanins	Vitaglione et al. (2007)
5% HAc in water	MeOH	-	DAD, ESI-MS	68 min	RP	anthocyanins	Wu et al. (2002)
0.5% H_3PO_4	MeOH	-	DAD	23 min	RP	anthocyanins	Matsumoto et al. (2001)
5% HAc in water	5% HAc in ACN	-	DAD	35 min	RP	anthocyanins	Määttä et al. (2003)
0.5% H_3PO_4	MeOH	-	DAD	45 min	RP	anthocyanins	Jakobek et al. (2009)
10% HAc	ACN	-	DAD, ESI-MS	40 min	RP	anthocyanins	Ogawa et al. (2008)
2% EtAc in water	0.5% EtAc in water/ACN (1:1)	-	DAD, ESI-MS	65 min	RP	ellagitannins	Hager et al. (2010)
0.1% EtAc in water	ACN/MeOH (1:1)	-	DAD, ESI-MS	25 min	RP	flavan-3-ols, phenolic acids	Taruscio et al. (2004)
$NH_4H_2PO_4$, 50mM, pH2.6	20% A + 80% ACN	H_3PO_4, 200 mM, pH 1.5	DAD, FLD	86 min	RP	flavonoids	Lamuela-Raventos and Waterhouse (1994)
0.1% HAc in water	80% ACN in water	-	DAD, ESI-MS	55 min	RP	flavonoids	Fang et al. (2007)
3% EtAc in water	MeOH	-	DAD	47 min	RP	flavonoids	Chen and Zuo (2007)
0.1% H_3PO_4	ACN	-	DAD	60 min	RP	flavonoids	Lai et al. (2007)
Water, pH 1.35 with TFA	ACN, pH 1.35 with TFA	-	DAD	137 min	RP	flavonoids	Kalt et al. (2008)

[a] MeOH, methanol; TFA, trifluoric acetic acid; ACN, acetonitrile; HAc, formic acid; EtAc, acetic acid; [b] RP, reversed phase; NP, normal phase

Table 3. Continued

Solvents[a]			Detectors	Runtime	Phase[b]	Type of phenolics	Reference
A	B	C					
1% HAc in water	ACN	-	DAD, ESI-MS	60 min	RP	flavonoids	Borges et al. (2010)
1% HAc in water	ACN	-	DAD	32 min	RP	flavonols	Häkkinen and Törrönen (2000)
0.05% TFA in water, pH 2	ACN	-	DAD	31 min	RP	flavonols, anthocyanins	Taruscio et al. (2004)
0.1% H_3PO_4 in water	MeOH	-	DAD	35 min	RP	flavonols, flavan-3-ols, phenolic acids	Jakobek et al. (2009)
50 mM H_3PO_4, pH 2.5	ACN	-	DAD	67 min	RP	phenolic acids	Mattila et al. (2006)
1% HAc in water	ACN	-	DAD	37 min	RP	phenolic acids	Häkkinen and Törrönen (2000)
1% HAc in water	1% HAc in ACN	-	DAD, ESI-MS	20 min	RP	phenolic acids, flavonoids	Määttä et al. (2003)
1% HAc in water	ACN	-	DAD, ESI-MS	70 min	RP	phenolic acids, flavonoids	Seeram et al. (2006)
2% EtAc in ACN	ACN/water/EtAc (95:3:2)	-	DAD	45 min	NP	proanthocyanidins	Buendía et al. (2010)
MeOH	CH_2Cl_2	50% EtAc in water	APCI-MS, FLD	85 min	NP	proanthocyanidins	Kalt et al. (2008)
CH_2Cl_2/MeOH/water/ EtAc (41:7:1:1, v/v/v)	CH_2Cl_2/MeOH/ water/EtAc (5:43:1:1, v/v/v)	-	DAD, FLD	60 min	NP	proanthocyanidins	Hager et al. (2010); Hellström et al. (2007)

[a] MeOH, methanol; TFA, trifluoric acetic acid; ACN, acetonitrile; HAc, formic acid; EtAc, acetic acid; [b] RP, reversed phase; NP, normal phase

2.4 Radical scavenging and antioxidant activity testing *in vitro*

2.4.1 Radical scavenging and reducing methods

Different antiradical methods have been used to evaluate the antioxidant activity of phenolic compounds. These methods can be divided into two groups: assays based on hydrogen atom transfer reactions, and those based on electron transfer reactions (Huang et al. 2005). In these assays, reagents are already radicals, such as 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS+) or 2,2-diphenyl-1-picrylhydrazyl (DPPH), or they are formed during the assay (2,2´-Azo-bis(2-amidinopropane) dihydrochloride (AAPH)). In these methods, color formation or disappearance is followed by a spectrophotometer. Changes in colors are due to the radical quenching ability of the antioxidants (Nilsson et al. 2005; Jakobek et al. 2009). The antiradical results are usually plotted against a Trolox calibration curve to obtain the Trolox equivalent activity (TEAC). Trolox is a water-soluble derivative of vitamin E (Castrejón et al. 2008; Garzón et al. 2009). The disadvantage of these methods is that the radicals and probes used in these assays are not naturally present in foods (Frankel and Meyer 2000). In addition, the use of radical scavenging methods differs between laboratories, making the comparison difficult. **Table 4** summarizes the antiradical methods.

Oxygen radical absorbance capacity (ORAC) assay is often used for measuring the oxidative degradation of the fluorescent compound fluorescein (Cao et al. 1993; Kalt et al. 1999; Ou et al. 2001; Atala et al. 2009; Denev et al. 2010;). Degradation is induced by AAPH, a peroxyl radical generator, and the loss of fluorescence is measured. Trolox standard dilutions are used for calibration, and the results are expressed as Trolox equivalents. Samples (1:20 of total volume) are mixed with AAPH (4–50 mM) and fluorescein (5 x 10^{-8} – 1 x 10-5 M). ORAC methods can be easily automated, thus providing the opportunity for high-throughput methods.

Total radical-trapping parameter (TRAP) assay has been developed to measure the antioxidant capacity of plasma or serum. In this assay, peroxyl radicals are usually generated by 2,2'azobis(2-amidinopropane) hydrochloride (ABAP). Formed radicals oxidize antioxidants in plasma or serum, and the oxygen consumption is measured (Wayner et al. 1985). DeLange and Glazer (1989) modified the method by using R-phycoerythrin as a fluorescent probe. Peroxyl radicals quench the fluorescence, which is slowed down by antioxidants.

In the ABTS method, blue-green ABTS+ radical is first formed with potassium persulfate. The discoloration of ABTS+ is followed by a spectrophotometer at 730–734 nm for 6 minutes. Samples (100 uL) are mixed with ABTS+ (0.5–1.0 mL). The calibration curve is prepared from Trolox and the antioxidant activity is expressed as Trolox equivalents (Miller et al. 1995; Nilsson et al. 2005; Castrejón et al. 2008; Garzón et al. 2009; Marques et al. 2010).

The ferric-reducing antioxidant power (FRAP) method is based on the ability of phenolic compounds to reduce ferric chloride (Benzie and Strain 1996; Benzie and Strain 1999). To 900 uL of FRAP reagent (10 mM 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ), 20 mM $FeCl_3$, 0.1–0.3 mM acetate buffer), 30 uL of samples and 90 uL of water are added (Nilsson et al. 2005; Garzón et al. 2009); alternative, 30 uL of 1:40 diluted samples are added to 250 uL of FRAP solution (Hukkanen et al. 2006). The color change from yellow to blue is observed at 593–595

nm. The FRAP method does not react to antioxidants with thiol structures such as glutathione; it only reacts to ferric ion–based assays (Prior et al. 2005).

The advantage of the DPPH method is its rapidity and simplicity. DPPH is a purple-colored stable radical that is quenched by phenolic compounds to form a yellow color. The color change is measured by a spectrophotometer at 517 nm. Methanolic samples are mixed with 0.1 mM of DPPH solution. The calibration curve is made from diluted samples to determine the median effective concentration EC_{50} value, the amount of phenolic compounds needed to reduce the DPPH radical by 50% (Brand-Williams et al. 1995; Benvenuti et al. 2004; Hukkanen et al. 2006). Another way to express the results is to determine the percent inhibition, that is, the percentage of DPPH radical reduced (Kähkönen and Heinonen 2003). DPPH discoloration can occur via either a hydrogen atom transfer reaction or an electron transfer reaction. In addition, steric accessibility determines the rate of the reaction. Thus, small molecules that have better access to the radical site have higher antiradical activity in this test (Prior et al. 2005).

In cupric reducing antioxidant capacity (CUPRAC) assay, hydrogen peroxide–derived radicals are quenched by the phenolic compounds. Due to the low absorption maximum of hydrogen peroxide (230 nm), copper(II)-neocuproine (Cu(II)-Nc) is added to the reaction mixture as a chromogenic agent. After incubation for 30 minutes, the absorbance is measured at 450 nm (Apak et al. 2008).

Table 4. Radical scavenging and reducing methods (adapted from Hollman et al. 2011).

Assay	Principle	Oxidant	Probe	Detection	Application	Main limitations
Hydrogen atom transfer reactions						
TRAP	chain-breaking capacity	ROO•	R-PE	fluorescence (495/575 nm)	foods, body fluids, and tissues	lipophilic antioxidants not included, non-physiological probe
ORAC	chain-breaking capacity	ROO•	B-PE or FL	fluorescence (495/575 nm)	foods, body fluids, and tissues	may be modified to include lipophilic antioxidants, protein interferences, non-physiological probe
Electron transfer reactions						
FRAP	reducing capacity	TPTZ-Fe^{3+}	TPTZ-Fe^{3+}	absorbance (595 nm)	foods, plasma	thiols not included, non-physiological probe
TEAC	reducing capacity	$ABTS^{\bullet+}$	$ABTS^{\breve{y}+}$	absorbance (734 nm)	foods, body fluids	low-sensitivity, non-physiological probe
DPPH•	reducing capacity	DPPH•	$DPPH^{\breve{y}}$	absorbance (517 nm)	foods	non-physiological probe
CUPRAC[a]	reducing capacity	H_2O_2	Cu(II)-Nc	absorbance (450 nm)	foods	non-physiological probe

[a] Apak et al. 2008; R-PE: R-phycoerythrin, B-PE: R-phycoerythrin, FL: fluorescein, TPTZ-Fe^{3+}: ferric 2,4,6-Tris(2-pyridyl)-1,3,5-triazine, $ABTS^{\breve{y}+}$: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid, $DPPH^{\breve{y}}$: 2,2-diphenyl-1-picrylhydrazyl, Cu(II)-Nc: copper(II)-neocuproine

2.4.2 Food models

Foods are complex systems comprising various oxidizing components such as lipids and proteins. Due to the complexity of the foods, it is necessary to consider the antioxidant activity test conditions. The use of simplified, one-dimensional methods that utilize nonspecific substrates to evaluate the antioxidant activity in complicated systems makes the interpretation of the results difficult. Therefore, food model systems should be applied when the antioxidant activity of foods are measured. Frankel and Meyer (2000) proposed a testing protocol to evaluate multifunctional food and biological antioxidants. They argued that, since the effectiveness of antioxidants is dependent on the test, the substrate should be relevant to the test. In addition, antioxidant activity testing should be conducted in different oxidation systems, and both primary and secondary oxidation products should be measured.

Bulk oil

Bulk oil has been a model system to study the antioxidant activity in hydrophobic lipid oxidation systems (Frankel et al. 1994; Frankel et al. 1996; Huang et al. 1996; Pekkarinen et al. 1999). Porter et al. (1993) observed that polar antioxidants were more active in bulk oils than in aqueous lipid systems such as emulsions. On the other hand, lipophilic antioxidants have been observed to be more effective in emulsions than in bulk oil. This phenomenon is known as polar paradox, and several studies have proven it (Porter et al. 1993; Frankel et al. 1994; Frankel et al. 1996; Huang et al. 1996; Pekkarinen et al. 1999). Ascorbic acid and Trolox were effective preventing the oxidation of bulk oil, while α-tocopherol was weaker (Frankel et al. 1994). Kähkönen et al. (2003) found that more hydrophilic anthocyanin glucosides were better antioxidants than their aglycons in bulk oil oxidation assay.

Emulsion

Emulsions are formed of oil droplets in a continuous water phase, and they represent a major group of colloidal food systems (Schwarz et al. 2000). Partition of active compounds into different phases of the emulsions is important. Hydroxycinnamic acids, caffeic acid, ferulic acid, and sinapic acid were solubilized into water-oil biphasic and emulsion systems. These acids showed higher solubilization into the lipid phase of the emulsions than into the water-oil system. This solubilization has a significant role, since the antioxidant activity may be enhanced due to the proximity of the oxidizable lipids (Pekkarinen et al. 1999). Compounds adsorbing in the oil-water interface may stabilize emulsions by preventing droplets from shrinking or coalescing. The flavonol rutin and flavanone naringin showed such a behavior in an emulsion (Luo et al. 2011). Moreover, Viljanen et al. (2005) studied the partitioning behavior of the anthocyanin fractions of blackcurrants, raspberries, and lingonberries in oil-in-water emulsion with whey protein isolate as an emulgator. In this study, the major part of the anthocyanins was located in the aqueous phase, and only a fraction in the oil-water interface. Blackcurrant anthocyanins inhibited oxidation of the protein-containing emulsion at 100 and 500 μg/g concentrations, while anthocyanins from raspberries and lingonberries displayed this inhibition only at concentrations of 500 μg/g. In a methyl linoleate emulsion, bilberry,

blackcurrant, and lingonberry anthocyanin fractions had a similar antioxidant activity to that reported in whey protein emulsions (Kähkönen et al. 2003). Proanthocyanidin fractions from bilberries, lingonberries, and cranberries were also effective in retarding emulsion oxidation, whereas bog whortleberry proanthocyanidin fractions were not as effective (Määttä-Riihinen et al. 2005).

Meat model

Lipid oxidation is the main cause of deterioration of meat and muscle foods, leading to a loss of sensory and nutritional value. Lipid oxidation occurs in the presence of reactive oxygen species (ROS). Primary oxidation products, hydroperoxides, are unstable, and decompose easily into secondary oxidation products such as aldehydes. Unstable hydroperoxides or ROS may oxidize susceptible amino acids (amino or sulfhydryl side chains), leading to the formation of carbonyl compounds or cross-links (Vuorela et al. 2005; Salminen et al. 2006; Ganhão et al. 2010b). The measurement of carbonyl compounds and volatile aldehydes, mainly hexanal, can be used for the estimation of the meat lipid and protein oxidation (Rey et al. 2005; Vuorela et al. 2005; Salminen et al. 2006). Phenolic compounds have been shown to reduce the oxidation of meat lipids and proteins. Usually, pork meat has been used for the evaluation of antioxidant activity of plant phenolic compounds, but turkey or fish meat has also been used (He and Shahidi 1997; Mercier et al. 1998; Rey et al. 2005; Vuorela et al. 2005; Salminen et al. 2006; Haak et al. 2009; Ganhão et al. 2010a; Ganhão et al. 2010b). Salminen et al. (2006) found that rapeseed and camelina meals and their combination with rosemary extract inhibited hexanal formation excellently in a pork meat oxidation assay. In another study, rosemary extract was also found to inhibit lipid peroxidation (Haak et al. 2009). Vuorela et al. (2005) came to in the same conclusion with aqueous ethanolic and pectinolytic enzyme–assisted extracts of rapeseed meals and pine bark extract. Extracts of the Mediterranean fruits of the strawberry tree, common hawthorn, dog rose, and elm-leaf blackberry were shown to exhibit strong antioxidant activity toward lipid oxidation in a pork meat model (Ganhão et al. 2010a). These Mediterranean fruits were found to be rich in proanthocyanidins. Cloudberries, abundant in ellagitannins, were also noted to inhibit the hexanal formation of cooked pork meat patties (Rey et al. 2005).

2.4.3 Biological models

In biological systems, an antioxidant can be defined as a substance that presents at low concentrations compared to the oxidizable substrates, i.e., lipids, can remarkably prevent oxidation (Frankel and Meyer, 2000). Liposomes, phospholipid bilayers, mimicking biomembranes, and low-density lipoproteins (LDL) have been used as models for biological oxidation systems.

Liposomes

Lecithin liposomes consist of a charged phosphatidylcholine bilayer enabling them to be better models for hydrophilic antioxidants than emulsions (Huang and Frankel 1997). Heinonen et al. (1998) tested berry phenolic extracts in a liposome oxidation assay. Sweet cherries were more active compared to blackberries, blueberries, raspberries, and strawberries. The differences between cherries and other berries were in the phenolic content; cherries were rich in hydroxycinnamates and other berries rich in anthocyanins. The liposomes were prepared from lecithin containing 40% phosphatidylcholine as an oxidative lipid, and the oxidation was induced by cupric acetate. Vuorela et al. (2004; 2005) tested rapeseed meal and oil extracts for their antioxidant activity in the same model. Both berry and rapeseed extracts strongly inhibited the lipid oxidation in liposomes. Viljanen et al. (2004a: 2004b) modified the method by adding proteins into the liposomes to study protein oxidation and the interaction between proteins and oxidized lipids. The proteins used in these studies were lactalbumin, casein, and bovine serum albumin (BSA). Proteins, especially casein, stabilized liposomes and inhibited the lipid oxidation. The addition of anthocyanins, proanthocyanidins, or berry extracts had a preservative effect, inhibiting mostly lipid oxidation, but also to a lesser extent protein oxidation. Bilberry, blackcurrant, and red raspberry anthocyanins and lingonberry proanthocyanidins were found to be to most effective in the inhibition of lipid oxidation, while bilberry and blackcurrant phenolic extracts, red raspberry ellagitannins, and lingonberry anthocyanins were only moderate (Viljanen et al. 2004a).

LDL oxidation

The oxidation of LDL has been shown to have a role in the pathogenesis of atherosclerosis and coronary heart diseases (Lapointe et al. 2006). Oxidants, reactive oxygen species (ROS) originating from inflammation, exercise, the respiratory chain or smoke, pollutants, and UV light oxidize LDL particles, whereas antioxidative phenolic compounds protect LDL from oxidation (Heinonen et al. 1998; Meyer et al. 1998; Kähkönen et al. 2001; Kähkönen and Heinonen 2003; Määttä-Riihinen et al. 2005; Vuorela et al. 2005; Tuberoso et al. 2010; Singh et al. 2011). In these assays, the oxidation of LDL particles was initiated with copper. After 2 h incubation at +37 °C, the hexanal formation was measured as an indicator of oxidation. Phenolics from blackberries, blueberries, red raspberries, and sweet cherries inhibited the LDL oxidation well (Heinonen et al. 1998). Individual anthocyanidins and their glycosides, as well as caffeic acid, chlorogenic acid, catechin, quercetin, and rutin showed strong inhibition against LDL oxidation (Kähkönen and Heinonen 2003). In this study, aglycon forms of anthocyanins were more effective than their glycosylated forms. Blackberry, bilberry, and lingonberry anthocyanin fractions were also found to have a high antioxidant activity against LDL oxidation (Kähkönen et al. 2003). Vuorela et al. (2004) found the phenolic compounds in rapeseeds, sinapine being the main contributor, exhibit strong antioxidant activity toward LDL oxidation.

3 OBJECTIVES OF THE STUDY

The main objectives of this study were to isolate and identify different phenolic compounds in berries and investigate the antioxidant activity of these phenolic compounds toward lipid oxidation in different oxidation models.

The specific aims of the study were:

- to isolate and identify the phenolic compounds in wild and cultivated rowanberries
- to isolate and characterize the proanthocyanidins of cranberries and lingonberries and the ellagitannins of cloudberries
- to test the storage stability of the ellagitannin fraction of cloudberries
- to evaluate the antioxidant potential of pure phenolic compounds and phenolic extracts and fractions obtained from berries in liposome and emulsion oxidation models and in $DPPH^{\cdot}$ radical scavenging assay.

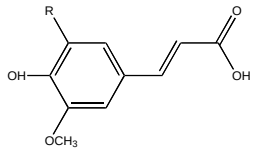
4 MATERIALS AND METHODS

4.1 Berry samples and phenolic compounds

Berries that differed in their phenolic profiles were chosen for this study (**Table 5)**. Phenolic composition and the antioxidant activities of cloudberry ellagitannins (**III, V**), wild rowanberry and cultivated sweet rowanberry phenolic extracts (**I**), and cranberry and lingonberry phenolic extracts and proanthocyanidins fractions (**II**) were studied (**Table 5**). Berries were purchased from the grocery stores and local marketplaces in Southern Finland. The cultivated rowanberries were obtained directly from the grower. The synthesized sinapic acid and ferulic acid and their glycosides used in study **IV** are shown in **Figure 2**.

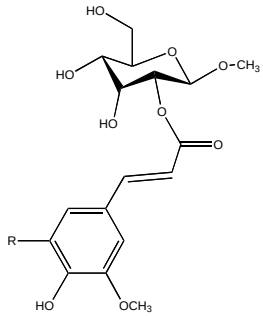
Table 5. Berries used in the studies.

Berry
Wild rowanberry *(Sorbus aucuparia*)
Cultivated rowanberries
Burka (*Sorbus aucuparia* × [*Sorbus aria* × *Aronia arbutifolia*])
Granatnaja (*Sorbus aucuparia* × *Crataegus sanguinea*)
Titan (burka × *Malus* sp. × *Pyrus* sp.)
Zoltaja (*Sorbus aucuparia* × *Pyrus* sp.)
Cranberry (*Vaccinium microcarpon*)
Lingonberry (*Vaccinium vitis-idaea*)
Cloudberry (*Rubus chamaemorus*)

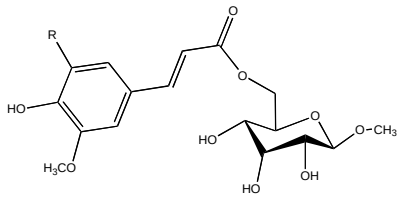


	R
Feruloyl	H
Sinapoyl	OCH_3

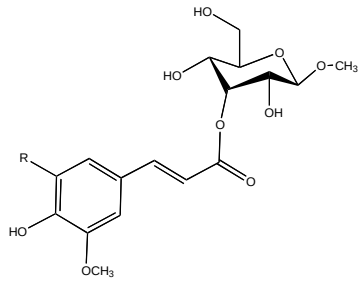
Hydroxycinnamic acid



Methyl 2-*O*-cinnamoyl-α-D-glucopyranoside



Methyl 6-*O*-cinnamoyl-α-D-glucopyranoside



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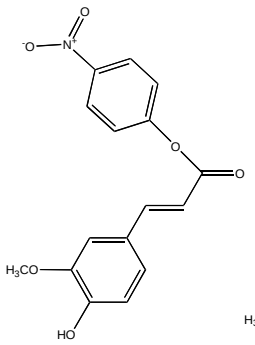
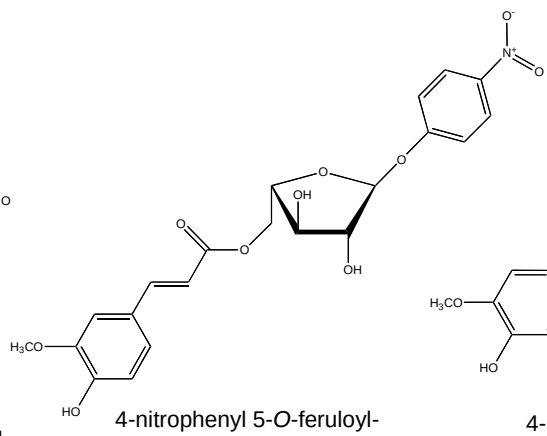
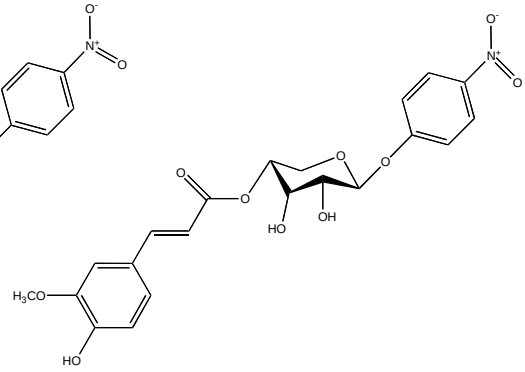


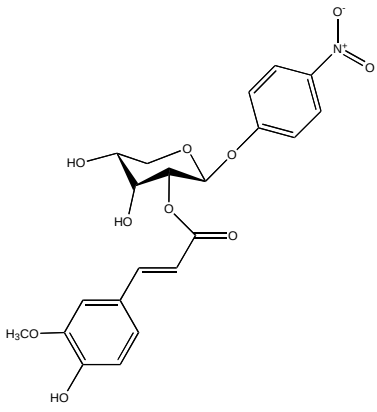
4-nitrophenyl ferulic acid



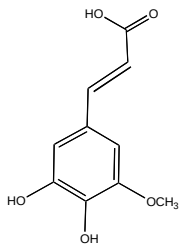
4-nitrophenyl 5-*O*-feruloyl-α-arabinofuranoside



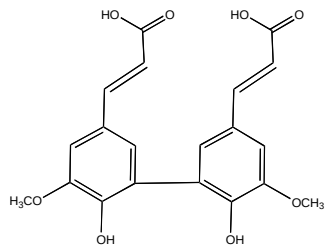
4-nitrophenyl 4-*O*-feruloyl-α-xylopyranoside



4-nitrophenyl 2-*O*-feruloyl-α-xylopyranoside



5-OH ferulic acid



Ferulic acid dehydrodimer

Figure 2. Structures of synthesized hydroxycinnamic acids and their glycosides (Study **IV**).

4.2 Extraction and analytical methods

4.2.1 Extraction and fractionation

Berry phenolics were extracted and fractionated into different fractions according to **Figure 3**. The extraction started from frozen and lyophilized berries. To isolate phenolics, anthocyanins, and ellagitannins, the extraction was performed with 70% aqueous acetone. For the proanthocyanidin isolation, the extraction was performed first with 70% aqueous acetone and subsequently with methanol using accelerated solvent extraction (ASE). Amberlite XAD-7 column chromatography and C-18 solid phase extraction (SPE) were used in order to remove sugars and organic acids. For further fractionation, Sephadex LH-20 column chromatography was used.

Solvent extraction

The isolation of phenolic compounds in studies **I** and **III-V** was carried out in triplicate as follows: 2.0–3.0 g freeze-dried berry material was weighed into the centrifuge tube as 6 replicates, 20 mL of 70% aqueous acetone was added, and the sample was homogenized with Ultra-Turrax for 1 min. Samples were centrifuged (1570 g, 15 min) and the supernatants were collected. The procedure was repeated with another 20 mL of 70% aqueous acetone. Supernatants were combined, evaporated to dryness with a rotary evaporator, and dissolved in 15 mL of water.

ASE for proanthocyanidin isolation

An ASE 200 System (Dionex, Sunnyvale, CA, USA) with 11 mL stainless steel ASE vessels was used for the ASE (**II**). About 1.0 g of freeze-dried berry material was mixed homogeneously with 0.25 g of diatomaceous earth and placed into an extraction cell. A total of 10 cells were used for extraction. The extraction was performed with 70% aqueous acetone and methanol. ASE settings were: pressure 1500 psi, temperature 100 °C, heat time 5 min, static time 5 min, 1 static cycle. Each sample was extracted twice. After this, the extraction supernatants were evaporated to dryness and dissolved in 5 mL of methanol.

Fractionation of phenolic extracts

Amberlite XAD-7 sorbent was used to remove sugars and organic acids from the crude phenolic extract prior to separating the ellagitannins and anthocyanins from other phenolic compounds (**III** and **V**). First, 10 g of XAD-7 sorbent was suspended in acetone and then packed into a glass column (300 x 40 mm) with 50% aqueous acetone and water. To condition the column, 0.5% trifluoroacetic acid in acetonitrile was applied to the column, followed by water, and finally 0.1% trifluoroacetic acid in water. Crude extract in water was added to the column. Sugars and organic acids were eluted with 6% aqueous acetonitrile. One hundred percent acetonitrile was used to elute the phenolic fraction. The extract was evaporated to dryness and dissolved in methanol.

Solid phase extraction (SPE) was carried out using C18 cartridges (5 g) (**I** and **II**). First, the cartridge was conditioned with 25 mL of methanol and then rinsed two times with 25 mL water. After conditioning, the sample in water (5 mL) was applied to the cartridge. To elute sugars and organic acids, 20 mL of 0.01 M HCl was added. The phenolic fraction was then eluted with 25 mL of methanol.

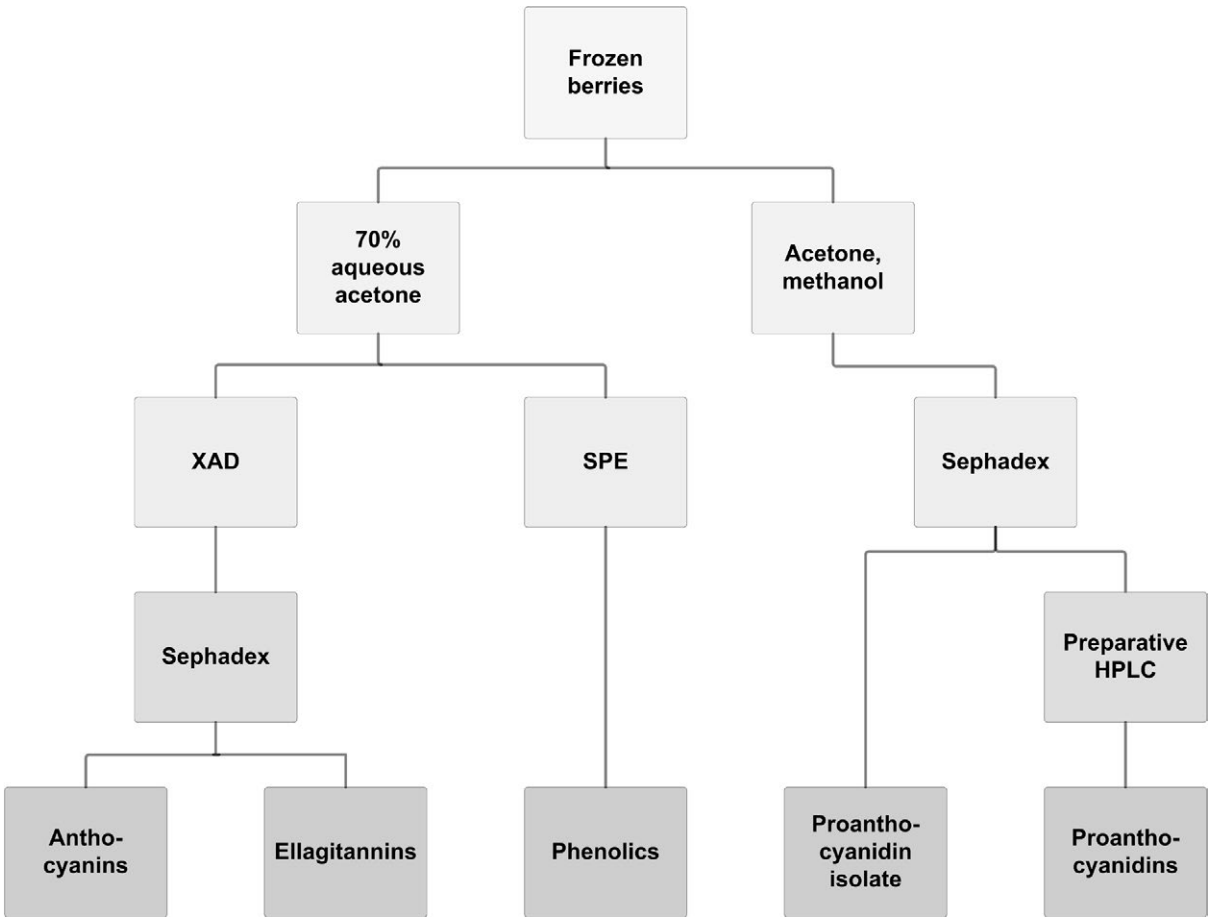


Figure 3. Fractionation scheme of berry phenolics.

Sephadex LH-20 column chromatography was used for the fractionation of ellagitannins, anthocyanins, and proanthocyanidins from other phenolic compounds (**II**). Sephadex LH-20 (20 g) was suspended in 100% acetone then packed into the column (300 x 40 mm) with 70% aqueous acetone and 50% aqueous methanol. To separate ellagitannins from anthocyanins, the extract obtained from XAD column chromatography was applied to the Sephadex column. Anthocyanins were eluted with 50% aqueous methanol and ellagitannins with 70% aqueous acetone. Both fractions were evaporated to dryness and dissolved in water.

To separate proanthocyanidins from other phenolic compounds, the extract obtained from accelerated solvent extraction was applied to the column, eluted with 50% aqueous methanol and then with 90% aqueous acetone. The methanolic fraction contained other phenolic compounds, while the proanthocyanidins were in the acetone fraction. Proanthocyanidins were evaporated to dryness and dissolved in acetonitrile and further fractionated by preparative HPLC.

4.2.2 Identification and HPLC quantification of berry phenolics

An HPLC system combined with a diode array detector was used for UV-Vis spectral identification and quantification. The identification of the phenolic compounds was based on the standard compounds and their similarities in the UV-Vis spectra. Compounds having similar spectra and peak maxima were grouped into hydroxybenzoic acids, flavan-3-ols, hydroxycinnamic acids, ellagitannins, flavonols, and anthocyanins and quantified as equivalents of known standard compounds. The standard compounds used for the calibrations were: gallic acid (hydroxybenzoic acids), (+)-catechin (monomeric flavan-3-ols), procyanidin B2 (dimeric, oligomeric, and polymeric flavan-3-ols), chlorogenic acid (hydroxycinnamic acids), ellagic acid (ellagitannins and ellagic acid), rutin (flavonols), and cyanidin-3-glucoside (anthocyanins). Known components—(-)-epicatechin, *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid, and quercetin—were quantified by using authentic standard compounds. LC-MS systems were used for qualitative analysis and tentative identification. Tentative identification was based on the literature and the fragmentation patterns of the compounds in the MS/MS experiment.

The quantification was done by external calibration. Calibration curves were prepared from the standard compounds listed above. Validation parameters for the methods used in studies **I** and **II** are listed in **Table 6**. Other methods were validated in previous studies. Recoveries of the standard compounds were tested by spiking a berry extract. The use of ultra performance liquid chromatography (UPLC) decreased the run time from 57 minutes to 28 minutes. The limits of detections and quantitations of the UPLC method were 3–16 times lower when compared to the HPLC method. For flavan-3-ols and proanthocyanidins, the limits of detection and quantitation were even better. The linearity and the range of quantitation were also improved with the UPLC. In general, recoveries in both methods were good. The recovery of anthocyanin cyanidin-3-glucoside was low in both methods, which may cause underestimation of the anthocyanin results. The recovery of ellagic acid was high (140%), causing overestimation of ellagic acid contents in the results.

HPLC conditions

To analyze and quantitate the phenolic compounds in berries, the following systems were used. In studies **I, IV-V**, the Waters Alliance (Waters, Milford, MA) chromatographic system was used (**Table 7**). This consisted of a Waters Alliance 2690 Separation Module, Waters PDA 996 diode array detector, and Waters 474 fluorescence detector coupled with the Empower 2 data-handling system. HPLC separation of phenolic compounds was achieved on a Waters Nova-Pac C_{18} (150 × 3.9 mm, 4 μm) column (**IV, V**) or Waters Atlantis T3 C_{18} (150 × 4.6 mm, 3 μm) column (**I**) protected with a guard column of the same material.

In studies **II** and **III**, the Waters ACQUITY UPLC (Waters, Milford, MA) system was used, which consisted of a binary solvent manager, sample manager, column heater, eλ PDA diode array, and FLD fluorescence detectors. The UPLC separation was achieved on a Waters HSS T3 C_{18} (150 × 2.1 mm, 1.8 μm) column (**Table 7**). Waters Empower 2 build 2154 was used to control the HPLC and UPLC systems and data handling.

Table 6. UPLC (**II**) and HPLC (**I**) method validation parameters.

Component	Retention time (RSD %)[a]	Peak area (RSD %)	Range µg/mL	Correlation coefficient R^2	LOD[a] µg/mL	LOQ[a] µg/mL	Recovery (%)
Study **II**							
Gallic acid	0.04	0.8	0.32-306.6	1.0000	0.10	0.32	102
Caffeic acid	0.05	3.0	0.17-163.0	0.9998	0.05	0.17	112
Chlorogenic acid	0.03	2.0	0.23-193.8	1.0000	0.04	0.23	101
Ferulic acid	0.02	1.7	0.19-197.8	0.9995	0.04	0.19	104
Sinapic acid	0.03	2.3	0.31-257.2	0.9998	0.04	0.31	126
p-coumaric acid	0.01	1.2	0.17-166.8	0.9997	0.05	0.17	100
Cyanidin-3-glucoside	0.02	1.2	1.87-94.0	0.9997	0.68	1.87	95
Catechin	0.01	0.7	0.05-148.6	0.9993	0.02	0.05	89
Procyanidin B2	0.01	0.6	0.19-193.6	0.9980	0.05	0.19	103
Rutin	0.01	0.6	2.13-47.9	1.0000	0.86	2.13	100
Ellagic acid	0.01	2.0	0.45-81.7	0.9998	0.25	0.45	140
Study **I**							
Gallic acid	0.01	1.9	1.20-20.0	0.9998	0.54	1.20	90
Caffeic acid	0.01	5.8	1.53-21.5	0.9997	0.67	1.53	111
Chlorogenic acid	0.01	9.2	1.90-24.9	0.9999	0.66	1.90	114
Ferulic acid	0.02	6.8	1.48-39.7	0.9998	0.46	1.48	116
Sinapic acid	0.01	5.1	1.50-21.6	0.9998	0.63	1.50	91
p-coumaric acid	0.01	6.2	1.25-20.0	0.9997	0.51	1.25	117
Cyanidin-3-glucoside	0.08	1.0	6.16-306.6	0.9999	2.48	6.16	81
Catechin	0.30	2.9	11.85-20.2	0.9994	3.55	11.85	100
Procyanidin B2	0.24	9.1	36.53-179.6	0.9992	12.18	36.53	124
Rutin	0.06	2.6	5.88-20.4	0.9999	2.25	5.88	107
Ellagic acid	0.07	2.7	7.26-9.3	0.9973	2.28	7.26	118

[a] RSD, relative standard deviation; LOD, limit of detection; LOQ, limit of quantification; n = 6

LC-MS identification of rowanberry phenolics (**I**)

To identify the rowanberry phenolics, the LCQ-Deca (Thermo Electron Corp., Waltham, MA) system was used (**Table 7**). The apparatus consisted of a Surveyor autosampler, pump, diode array detector (DAD), and a ThermoFinnigan ion trap mass spectrometer. Analyses were performed in the positive and negative mode. In the positive mode, the source voltage was set to 5000 V and the cone voltage to 46 V. In negative mode, the source voltage was set to -5000 V and the cone voltage to -44 V. In both modes, the capillary temperature was 275 °C; sheath gas flow 60.0 L/min; and auxiliary gas flow 25.0 L/min. Xcalibur 1.3 was used for MS data handling.

LC-MS identification of cranberry and lingonberry phenolics (**II**)

Agilent 1100 LC instrument, including diode array and fluorescence detectors, equipped with Bruker Esquire-LC ion trap ESI-MS (Bruker Daltonics, Bremen, Germany) was used for the characterization of cranberry and lingonberry proanthocyanidins. LC conditions were the same as in the quantitative analysis in study **I** (**Table 7**). The eluent flow was split to 1:10. ESI-MS analyses were performed in positive mode and the parameters were as follows: dry gas 10.0 L/min, nebulizer 60 psi, ESI interface temperature 350 °C, capillary voltage 3 kV, end-plate offset -500 V, skimmer 1 15.0 V, skimmer 2 6.0 V, lens 1 – 5.0 V, lens 2 – 60 V, trap drive value 45.0. MS data was analyzed by an LC/MSD Trap 5.2.

Preparative HPLC and LC-MS identification of cranberry and lingonberry proanthocyanidins (**II**)

A Waters semi-preparative HPLC (Waters 2767 sample manager, Waters 2545 binary gradient module and, Waters system fluidic organizer) was coupled with a Waters 2998 DAD, Waters 474 fluorescence detector, and Waters ZQ mass spectrometer to fractionate proanthocyanidins into several subsamples. Each subsample was further analyzed with LC-MS after the thioacidolysis. Subsamples were then pooled to dimers and trimers, oligomers, and polymers. Chromatographic separation was performed with YMC-Pack Diol-120-NP, 250 × 20 mm, 5 µm column (**Table 7**). The injection volume was 6 mL, and the flow rate 15 mL/min. The mass spectrometer settings were: capillary -2.80 kV, cone -32.00 V, Extractor -2.81 V, RF Lens -0.5 V, source temperature 120 °C, desolvation temperature 350 °C, cone gas flow 50 L/h, and desolvation gas flow 600 L/h. The system was controlled by Waters MassLynx 4.1 software.

Thioacidolysis of cranberry and lingonberry proanthocyanidins (**II**)

Cranberry and lingonberry proanthocyanidin fractions or procyanidin B1 and B2 standards (25 µL) were mixed with 35 µL 3.3% v/v HCl in methanol and 50 µL 10% benzylmercaptan in methanol. Mixtures were incubated in a water bath at 40 °C for 30 minutes. The quantitation was done by external calibration with catechin, epicatechin, and procyanidin B1 and B2.

LC-MS identification of cloudberry ellagitannins (**III**)

For the analysis of cloudberry ellagitannins, the Waters ACQUITY UPLC system connected to the Bruker Esquire-LC ion trap ESI-MS was used (**Table 7** studies **II** and **III**). ESI-MS analyses were performed in negative mode and the parameters were as follows: dry gas 10.0 L/min, nebulizer 60 psi, ESI interface temperature 300 °C, capillary voltage 3.5 kV, and end-plate offset -500 V. Smart tuning settings were: trap drive level 100%, compound stability 25%, and wide optimization. MS data were analyzed by LC/MSD Trap 5.2.

Table 7. Liquid chromatographic systems used in studies **I-V**.

Study	Column	Solvents		Gradient			DAD (nm)	FLD (nm)	Flow rate	Temperature
I	Waters Atlantis T3 C18 (150 × 4.6 mm, 3 µm)	A: B:	0.5% formic acid in water 0.5% formic acid in acetonitrile	0 min: 1 min: 5 min: 15 min: 25 min: 30 min: 33 min: 40 min: 43.5 min: 47 min: 50 min: 57 min:	100% A 100% A 93.5% A 90% A 84% A 84% A 76% A 68% A 36% A 36% A 100% A 100% A	0% B 0% B 6.5% B 10% B 16% B 16% B 24% B 32% B 64% B 64% B 0% B 0% B	280, 320, 365, 520	Ex 280 Em 325	1.0 mL/min	40 °C
I	Phenomenex Synergi Hydro C18 (150 × 2.0 mm, 4 µm)	A: B:	0.5% formic acid in water 0.5% formic acid in acetonitrile	0 min: 75 min:	95% A 60% A	5% B 40% B	280, 365, 520	-	0.4 mL/min	25 °C
II, III	Waters HSS T3 C18 (150 × 2.1 mm, 1.8 µm)	A: B:	0.5% formic acid in water 0.5% formic acid in acetonitrile	0 min: 1 min: 3.5 min: 9.8 min: 16 min: 19 min: 21 min: 23 min: 25 min: 27 min: 28 min: 31 min:	100% A 100% A 94% A 90% A 84% A 84% A 76% A 68% A 36% A 36% A 100% A 100% A	0% B 0% B 6% B 10% B 16% B 16% B 24% B 32% B 64% B 64% B 0% B 0% B	280, 320, 365, 520	Ex 280 Em 325	0.5 mL/min	40 °C

Table 7. Continued

Study	Column	Solvents	Gradient				DAD (nm)	FLD (nm)	Flow rate	Temperature
II	YMC-Pack Diol-120-NP (250 × 20 mm, 5 µm)	A: dichloromethane/methanol/ water (42:7:1) B: dichloromethane/methanol/ water (5:44:1)	0 min: 45 min: 65 min: 66 min: 70 min: 71 min: 73 min: 80 min:	100% A 70% A 70% A 15% A 15% A 70% A 100% A 100% A	0% B 30% B 30% B 85% B 85% B 30% B 0% B 0% B		280	-	15 mL/min	25 °C
IV	Waters Nova-Pac C18 (150 × 3.9 mm, 4 µm)	A: 20 mM $NH_4H_2PO_4$ buffer (pH 2.15) /methanol (75:25) B: Methanol	0 min: 15 min: 20 min: 35 min: 45 min: 50 min: 70 min:	95% A 95% A 65% A 65% A 0% A 95% A 95% A	5% B 5% B 35% B 65% B 100% B 5% B 5% B		280, 320	-	1.0 mL/min	30 °C
V	Waters Nova-Pac C18 (150 × 3.9 mm, 4 µm)	A: 50 mM $NH_4H_2PO_4$ (pH 2.60) B: Acetonitrile:solvent A (80:20) C: 0.2 M *o*-phosphoric acid (pH 1.5)	0 min: 5 min: 15 min: 25 min: 25.1 min: 45 min: 50 min: 55 min: 65 min: 70 min: 75 min: 90 min:	100% A 100% A 96% A 92% A 0% A 0% A 0% A 0% A 0% A 0% A 100% 100%	0% B 0% B 4% B 8% B 8% B 20% B 30% B 40% B 80% B 80% B 0% B 0% B	0% C 0% C 0% C 0% C 92% C 80% C 70% C 60% C 20% C 20% C 0% C 0% C	280, 320, 365, 520	Ex 280 Em 325	0.5 mL/min	40 °C

4.2.3 Spectrophotometric methods (IV)

A Perkin-Elmer λ 25 UV-vis spectrophotometer (Norwalk, CT) was used for the total phenolic content determination according to the Folin-Ciocalteau procedure at a wavelength of 765 nm. The absorbances were measured for DPPH· radical scavenging assay at the 517 nm wavelength, and for conjugated diene hydroperoxide at the 234 nm wavelength (**Chapter 4.3**). Total phenolic content of the ferulic acid and sinapic acid and their glycosides were determined by the Folin-Ciocalteau method. Samples (0.2 mL in methanol) were evaporated to dryness. After that, 0.2 mL of deionized water, 1 mL of Folin-Ciocalteu reagent (1:10), and 0.8 mL of disodium carbonate solution (7.5%) were added. After 30 min, the total phenolic content was measured at 765 nm by a Perkin-Elmer λ 25 UV-vis spectrophotometer (Singleton and Rossi 1965). Sinapic acid and ferulic acid were used as standards.

4.3 Encapsulation of cloudberry phenolics (V)

Maltodextrin DE5-8 or DE18.5 (9% w/w) and cloudberry phenolic extract (1% w/w) were dissolved into distilled water. The mixture was slightly heated (~50 °C) and stirred for 30 min. The pH of solutions was measured, and in all cases, it was 2.9. Solutions were pipetted into the 20 mL brown glass vials as a portion of 4.5 mL. Following this, the solutions were frozen at -20 and -80 °C for 2 and 19 h, respectively, and then placed into the freeze dryer (Lyovac GT2 freeze-dryer, Amsco Finn-Aqua GmbH, Hürth, Germany) and dried for 48 h (pressure <0.1 mbar). As a reference, phenolic extract–water solution without maltodextrin was freeze dried similarly. To prepare amorphous matrices without phenolics, maltodextrins (9% w/w) were dissolved in distilled water and solutions were freeze dried as described above. The dried samples were powdered using a glass rod and placed into vacuum desiccators over P_2O_5 (Merck, Darmstadt, Germany) for at least 1 week before further usage.

Unencapsulated cloudberry extract and microcapsules were stored in desiccators at 0, 33, and 66% relative vapor pressures (RVP) at 25 °C for 0, 16, 32, and 64 days. The storage RVP conditions represent the typical RVP values of room air during the winter (~30%) and summer (~60%) in Finland.

4.4 Radical scavenging and antioxidant activity tests

The antioxidant activities of phenolic compounds present in berries were evaluated in liposome and emulsion oxidation models, and antiradical activities in DPPH·radical scavenging assay (**Table 8**).

Table 8. Antioxidant or radical scavenging activity test design of studies **I-IV**.

Antioxidant or radical scavenging activity test	Experimental conditions	Antioxidants	Concentrations	Methods[a]	Study
Lecithin liposome model	+37 °C, 3 days, 3 μM CuAc	ferulic acid and sinapic acid glycosides, ferulic acid and sinapic acid standards, cloudberry phenolic extract, wild and cultivated rowanberry extracts, cranberry and lingonberry extracts and proanthocyanidin fractions	2.1, 4.2 and 8.4 μg/mL	CD, Hex	I, II, IV, V
Emulsion oxidation model	+37 °C, 6 days	ferulic acid and sinapic acid glycosides, ferulic acid and sinapic acid standards, wild and cultivated rowanberry extracts, cranberry and lingonberry extracts, and proanthocyanidin fractions	25, 50 and 100 μg/g	CD, Hex	I, II, IV
DPPH		ferulic acid and sinapic acid glycosides, ferulic acid and sinapic acid standards	1 mg/mL	Spectrometric method	IV

[a] CD, conjugated diene hydroperoxides; Hex, hexanal

4.4.1 Lipid oxidation (I-II, IV-V)

The lipid oxidation models chosen were phosphatidycholine liposomes (**I-II, IV-V**) and oil-in-water emulsion (**I-II, IV**). Liposomes were prepared from soybean lecithin (containing 40% phosphatidylcholine), and the concentration of phosphatidylcholine in samples was 0.8 mg/mL. Concentrations of the samples were 2.1, 4.2, and/or 8.4 μg/mL based on the content of total phenolics. Oxidation was started by adding 3 μM cupric acetate and the samples were incubated in shaking water bath (100 rpm, 37 ºC). The inhibition against liposome oxidation was calculated at day 3.

The emulsion (10 % o/w) oxidation model system (Kähkönen and Heinonen 2003) was prepared from purified rapeseed oil, water and Emultop, partially hydrolyzed soybean lecithin, and emulgator (2% w/w). α-Tocopherol (25 μM) was used as a reference antioxidant. Concentrations of the samples were 25, 50, and 100 μg/g oil based on the content of total phenolics. The samples were incubated at 37 °C for 6 days. The oxidation of lipids was followed by measuring conjugated diene hydroperoxides and hexanal. The antioxidant activity was expressed as inhibition against the formation of conjugated hydroperoxide dienes and hexanal, and was calculated as:

$$\text{inhibition (\%)} = (A_0 - A_1)/A_0 \times 100,$$

where A_0 is the absorbance for conjugated dienes or area for hexanal of a control sample and A_1 is the absorbance for conjugated dienes or area for hexanal of a tested sample.

Determination of conjugated diene hydroperoxides

A liposome sample (0.1 mL) or emulsion sample (0.025 mL) was dissolved in methanol (5 mL). The absorbance of the sample was measured at 234 nm, corresponding to hydroperoxides, with a Perkin Elmer λ 25 UV-vis spectrophotometer.

Determination of hexanal

For hexanal analysis, the sample of emulsion (0.25 mL) or liposome (0.5 mL) was transferred into a headspace vial and the formation of hexanal was measured by headspace gas chromatography. The headspace GC consisted of a Perkin Elmer Autosystem XL gas chromatograph equipped with flame ionization detector (Perkin Elmer, Shelton, CT), column NB-54 (5%-phenyl-1%-vinyl methyl polysiloxane–phase, 25 m × 32 μm) and Perkin Elmer HS40XL headspace autosampler. Column temperature was 60 °C, runtime 10 min, and the injector and detector temperatures were 250 °C. Samples were equilibrated for 18 min at 80 °C before the injection (**I-II, IV-V**).

4.4.2 DPPH radical scavenging test (IV)

The antiradical activity of ferulic acid and sinapic acid and their glycosides were determined according to method described by Malterud et al. (1993). Methanolic 0.1 mM 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) solution (2.95 mL) was mixed with 0.05 mL of the phenolic solution at the concentration of 1 mg/mL. The absorption was measured every 30 s for 4 min at the 517 nm wavelength with a Perkin-Elmer λ 25 UV-Vis spectrophotometer (Norwalk, CT). The results were expressed as the percentage of radicals scavenged in 4 min reaction time. The percentage of radical scavenging activity was calculated as:

radical scavenging activity (%) = $100 \times (A_1-A_2)/A_1$,

where A_1 is the initial absorbance at beginning of the reaction (t = 0) and A_2 is the absorbance after 4 min of reaction time.

4.5 Statistical analysis

In studies **I-V**, analyses were performed at least in triplicate and the differences between results were analyzed using one-way analysis of variance with Tukey's test. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA) or Statgraphics Plus 4.0 (Manugistics Inc., Rockville, MD, USA).

5. RESULTS AND DISCUSSION

5.1 Phenolic composition of berries

Phenolic compounds were extracted with 70% aqueous acetone from different berries. **Figure 4** shows the differences in the phenolic profiles of rowanberries, cranberries, lingonberries, and cloudberries. Rowanberry, belonging to the genus *Sorbus,* consists mainly of hydroxycinnamic acids; the phenolic fraction of genus *Vaccinium* species cranberry and lingonberry presents mostly flavan-3-ols, including proanthocyanidins; while cloudberry (genus *Rubus*) is rich in ellagitannins and ellagic acids. Proanthocyanidins, hydroxybenzoic acids, hydroxycinnamic acids, flavonols, and anthocyanins were found in all tested berries, while ellagitannins and ellagic acids were only present in cloudberries.

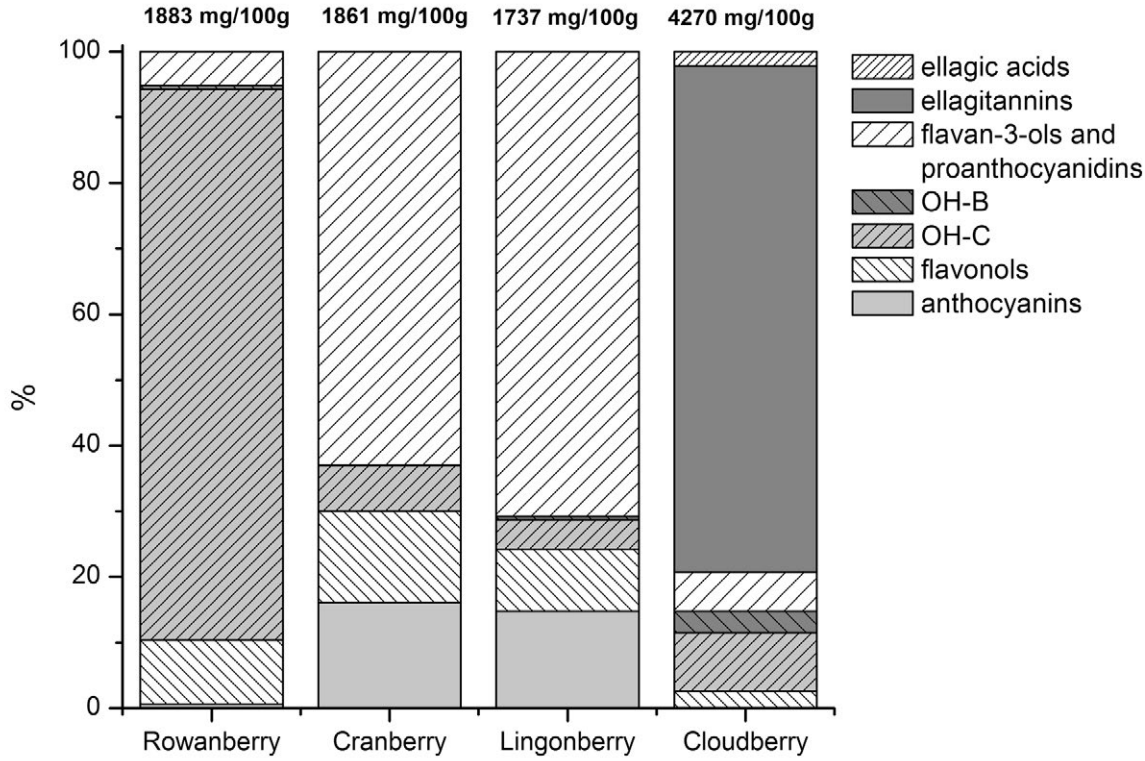


Figure 4. Phenolic profiles of rowanberries, cranberries, lingonberries, and cloudberries, OH-B: hydroxybenzoic acids, OH-C: hydroxycinnamic acids. Concentrations expressed as total amount of phenolics (mg/100g fw).

5.1.1 Rowanberries (I)

Chlorogenic acids (i.e., 3- and 5-caffeoylquinic acid) were the most abundant phenolic compounds in wild and cultivated rowanberries (**Figure 5** and **6**). Rowanberries were analyzed both in the negative and positive mode, with MS/MS identification data given in **Table 9**. In Zoltaja and wild rowanberry, chlorogenic acids comprised 75% of total phenolic composition. Some other quinic acid derivatives such as feruloyl and dicaffeoyl, quinic acids were also found. Cyanidin-3-galactoside was the main anthocyanin in all of the tested rowanberry cultivars.

Cyanidin-3-arabinoside was the other anthocyanin found. Proanthocyanidins up to hexamer were detected. All the flavonols determined were derived from quercetin.

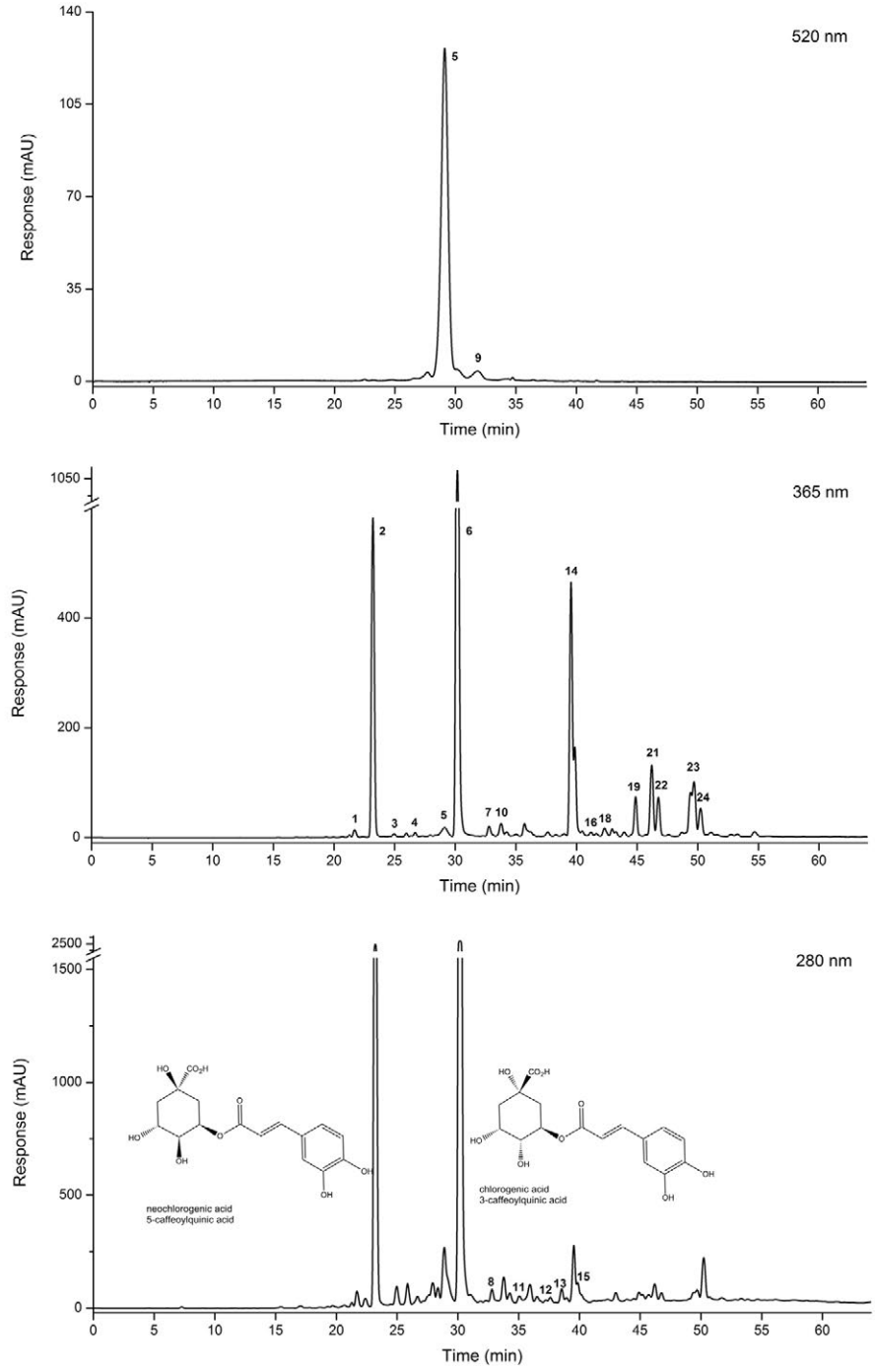


Figure 5. HPLC chromatogram of rowanberry phenolics at the 280, 365, and 520 nm wavelengths. Peaks 1–24 are listed in **Table 9**.

The cultivar Burka had the highest level of 5-caffeoylquinic acid at 7.31 mg/g dw (143 mg/100g fw) and Zoltaja the lowest at 2.23 mg/g dw (55 mg/100g fw); there was a threefold difference between them. Conversely, Zoltaja exhibited the greatest level of 3-caffeoylquinic (chlorogenic) acid (9.22 mg/g dw, 226 mg/100g fw), wild rowanberry being close behind at 8.59 mg/g dw (213 mg/100g fw), and Granatnaja having the lowest content at 3.20 mg/g (59

mg/100g fw). The wild rowanberry had the highest contents for total caffeoylquinic acids (5- + 3-), at 13.95 mg/g dw (346 mg/100g fw), while Granatnaja had the lowest, at 6.91 mg/g dw (128 mg/100g fw); thus, an almost threefold variation was evident across the samples analyzed.

Both anthocyanins and flavonols, as aglyconic and glycosidic forms, have been reported to occur in rowanberries (Häkkinen et al. 1999; Määttä-Riihinen et al. 2004; Olszewska 2008). In the present study, the range of flavonols in wild and cultivated rowanberries was 0.95–1.89 mg/g dw (23-36 mg/100g fw). The main phenolic group in Titan after hydroxycinnamic acids was anthocyanins at 6.04 mg/g (115 mg/100g fw), probably principally derived from chokeberry parentage. The levels of hydroxybenzoic acids were in the range of approximately 0.1–0.2 mg/g (3-4 mg/100g fw), except in Zoltaja, which contained 0.7 mg/g (17 mg/100g fw) of hydroxybenzoic acids.

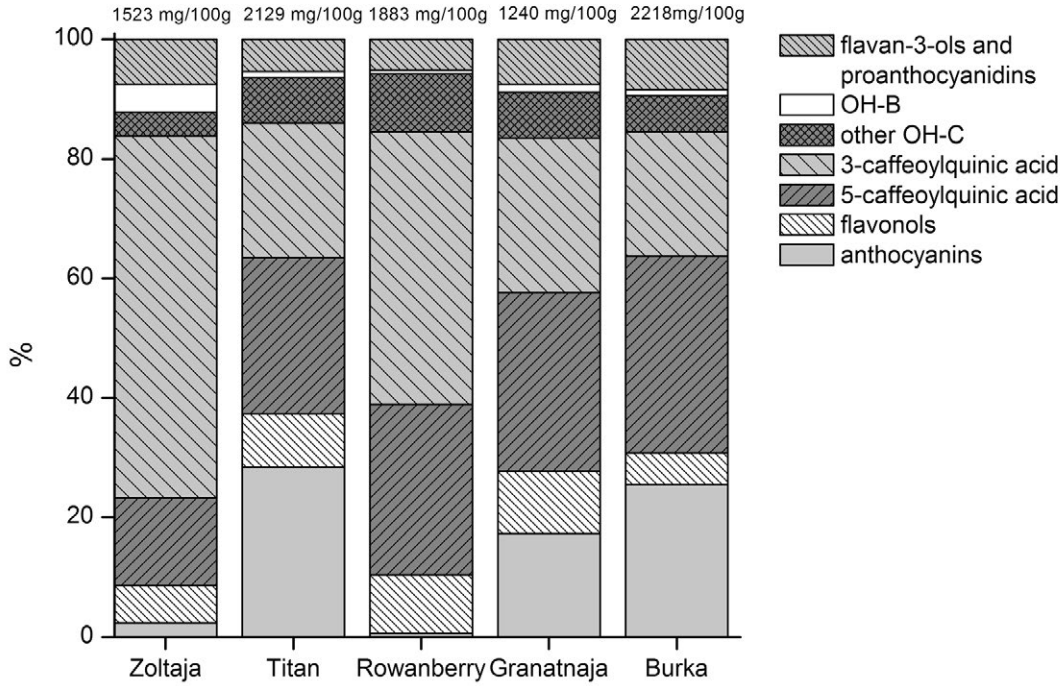


Figure 6. Phenolic composition of wild and cultivated rowanberries (Burka, Granatnaja, Titan, and Zoltaja). OH-B: hydroxybenzoic acids, OH-C: hydroxycinnamic acids. Concentrations expressed as total amount of phenolics (mg/100g fw).

5-Caffeoylquinic acid has been found in all crossing partners of sweet rowanberries, but neochlorogenic acid has only been reported for black chokeberry (*Aronia melanocarpa*) and wild rowanberries (Gil-Izquierdo and Mellenthin 2001; Määttä-Riihinen et al. 2004; Hukkanen et al. 2006). The levels of caffeoylquinic acids reported here are higher than those reported by Mattila et al. (2006) for the aglyconic caffeic acid, content for wild rowanberries (4.3 mg/g dw) and sweet rowanberries (*Crataegosorbus mitschurinii*) (3.1 mg/g). We also detected hydroxycinnamic acids other than 3- and 5-caffeoylquinic acids at 5–10 times higher levels than Hukkanen et al. (2006). Interestingly our data contradicts the finding of Häkkinen et

al. (1999) who reported that ferulic acid was the most abundant phenolic compound in wild rowanberries and the sweet rowanberry cultivar Granatnaja.

Hukkanen et al. (2006) analyzed the phenolic compounds in nine different sweet rowanberry cultivars. They reported the difference between the anthocyanin contents of Zoltaja (10 mg/100g fw) and Titan (101.6 mg/100g fw) was approximately 10-fold, and between Zoltaja (10 mg/100g fw) and Burka (156.5 mg/100g fw), there was a 15-fold difference. The corresponding values in the present study were approximately 12 and 13, respectively.

Table 9. Chromatographic, spectral, and mass features of phenolic compounds in wild rowanberry and sweet rowanberries after HPLC with DAD and MS^2 detection.

Peak	RT (min)	λ max	$[M+H]^+$ *m/z*	MS^2 fragments	$[M-H]^-$ *m/z*	MS^2 fragments	Tentative identity*
1	21.7	325	355	163	353	191	caffeoylquinic acid [a, b, c, e]
2	23.4	325	355	163	353	191	5-caffeoylquinic acid [a, b, c, d, e]
3	25.1	325			341	179	caffeoylglucoside [c]
4	27.2	315			515	353	dicaffeoylquinic acid [c, d]
5	28.5	515	449	287	447	285	cyanidin-3-galactoside [a, b, c, d, e]
6	30.4	325	355	163	353	191	3-caffeoylquinic acid [a, b, c, d, e]
7	32.4	325	369	163	367	191	feruloylquinic acid [c]
8	32.9	280			577	289	procyanidin dimer [b, c, d, e]
9	32.9	515	419	287	417	285	cyanidin-3-arabinoside [a, b, c, d, e]
10	33.9	315	355	163	353	191	caffeoylquinic acid [a, b, c, e]
11	35.2	280			289	245	catechin monomer [a, c, e]
12	37.3	280			865	575	procyanidin trimer [a, b, c, d, e]
13	38.6	280			1153	865	procyanidin tetramer [a, b, c, d]
14	39.6	355	627	303	625	301	quercetin dihexoside [a, b, c, d, e]
15	40.4	280			1441	1151	procyanidin pentamer [a, b, c]
16	41.4	325	369	163	367	191	feruloylquinic acid [c, e]
17	41.8	280			1729	1151	procyanidin hexamer [b]
18	42.4	345			595	301	quercetin hexoside pentoside [a, b, c, d, e]
19	45.3	355			609	301	quercetin-3-rutinoside [a, b, c, d, e]
20	45.5	325	465	289	463	287	eriodictyol glucuronide [a, b, d, e]
21	46.3	355	465	303	463	301	quercetin-3-hexoside [a, b, c, d, e]
22	46.7	355	465	303	463	301	quercetin-3-hexoside [a, b, c, d, e]
23	49.4	355			549	505, 301	quercetin malonylglucoside [a, b, c, d, e]
24	50.3	325			515	353	dicaffeoylquinic acid [a, b, c, d, e]

*[a] Burka, [b] Granatnaja, [c] Wild rowanberry, [d] Titan, [e] Zoltaja letters correspond to the cultivar the compound was found

In rowanberries, the main phenolic compounds were chlorogenic acids. Burka had the highest level of 5-caffeoylquinic acid, while Zoltaja, followed by the wild rowanberry, contained the most 3-caffeoylquinic acid.

5.1.2 Cranberries and lingonberries (II)

The main phenolic compounds in lingonberries and cranberries are proanthocyanidins, representing 71 and 63%, respectively, of the total phenolic compounds. Anthocyanins (15 and 16%), flavonols (9 and 14%), hydroxycinnamic acids (5 and 7%), and hydroxybenzoic acids (0.5 and 0.05%) were also found (**Figure 7**). In lingonberries, the contents of proanthocyanidins and hydroxybenzoic acids were higher than in cranberries, whereas cranberries contained more anthocyanins, flavanols, and hydroxycinnamic acids.

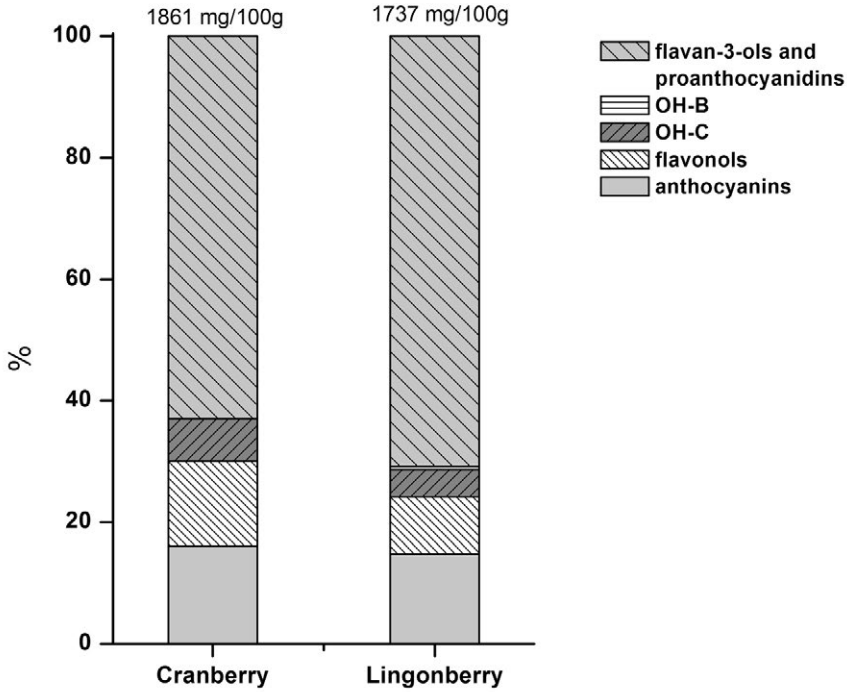


Figure 7. Phenolic profiles of cranberry and lingonberry extracts, OH-B: hydroxybenzoic acids, OH-C: hydroxycinnamic acids Concentrations expressed as total amount of phenolics (mg/100g fw).

The proanthocyanidins of cranberries and lingonberries were isolated and divided into subsamples by preparative HPLC (**Figure 7**). Subsamples were dimers and trimers, oligomers, and polymers. Thioacidolysis provided more detailed information on cranberry and lingonberry proanthocyanidin fractions. **Table 10** presents the structural composition of lingonberry and cranberry proanthocyanidin fractions as the percentages of moles. Catechin, epicatechin, A-type dimers, and A-type trimers were found as terminal units. The *m/z* values of the ions in the positive ion mode were 291, 291, 577, and 865, respectively, and the MS^2 fragmentations were similar to those described previously (Anderson et al. 2004; Karonen et al. 2007). The extention unit was detected as epicatechin benzylthioether, as it exhibited an $[M+H]^+$ ion at *m/z* 413 and an MS^2 fragment at *m/z* 289 on MS. Gallocatechin benzylthioethers ($[M+H]^+$ at *m/z* 429, MS^2 fragment at *m/z* 307) were also detected as an extension unit, but they were below the quantification limit.

The first fraction of cranberry and lingonberry contained dimers and trimers, as can be seen from the mean degrees of polymerization (mDPs), which were 2.4 and 2.3, respectively (**Table 10**). In lingonberries, the first fraction contained about 20% A-type dimers as terminal units, as well as catechins and only 3.4% of epicatechins. In cranberries, the proportion of catechins was the same as in lingonberries, but the content of epicatechin units was higher (12.1%). Cranberries also contained A-type trimers (7.9%) and less than 1% A-type dimers. The mDP of fractions 2-9 in lingonberries increased from 3.9 to 9.8, which is from tetramers to decamers. In cranberries, the trend was similar. The proportions of catechins and epicatechins in fractions 2–5 remained the same, and the mDP increased from 3.9 to 6.7. Fraction 5 contained oligomers from DP 6 to 10, as we were not able to fractionate it further. The quite low mDP of fraction 5 indicates a relatively low concentration of DPs 7–10. Overall, European cranberry contained more epicatechins and fewer A-type dimers as terminal units than lingonberry. In a previous study, lingonberries and European cranberries were noted to contain A-type dimers and trimers (Määttä-Riihinen et al. 2005). Our studies confirm the results of Hellström and Mattila (2008), showing that the proanthocyanidins of lingonberry contain significant portions of A-type linkages. The mDP of the polymeric fraction for lingonberry was 32.0 and 35.0 for cranberry. Both of these polymeric fractions comprised over 60% of the total proanthocyanidin content. The degree of polymerization of an American cranberry has been previously determined by Gu et al. (2002) to be 15, which is somewhat smaller than we detected.

Lingonberry fractions 2–9 were combined into one oligomeric fraction containing proanthocyanidins having mDP 4–10 and cranberry fractions 2-5 into another oligomeric fraction. Oligomeric fractions were combined in order to test the differences in the antioxidant activity between monomeric and dimeric, oligomeric, and polymeric fractions. Our assumption was that individual oligomeric proanthocyanidins do not differ from each other. Lingonberry fractions 1 and 10 and cranberry fractions 1 and 6 were kept as such (**Figure 8**).

In the present study, the phenolic profiles of lingonberries and cranberries were of the same order of magnitude and in accordance with the previous results obtained by Kähkönen et al. (2001) and Määttä-Riihinen et al. (2004), except for the flavanol and proanthocyanidin content in cranberries. Kähkönen et al. (2001) reported that the proanthocyanidin content of cranberries was 219 mg/100 g dw, while we found a content of 1.2 g/100 g dw. Määttä-Riihinen et al. (2004) also detected lower amounts of proanthocyanidins. This may be due to the ethyl acetate they used as an extraction solvent. The solubility of the higher molecular weight proanthocyanidins in ethyl acetate is lower than in acetone or methanol (Sun et al. 1998a). In another study, the proanthocyanidin content of the lingonberries was determined to be 158 mg/100 g fw (ca. 1000 mg/100 g dw), which is slightly less than reported here (Hellström and Mattila 2008). According to Mane et al. (2011), the main phenolic class in lingonberries is flavanols, followed by flavonols, phenolic acids, and anthocyanins, whereas we found that anthocyanins were the second most abundant phenolic class after flavanols. The main phenolic acids were *p*-coumaric acid and ferulic acid derivatives, and quercetin and quercetin-3-*O*-(3-hydroxy-3-methylglutaroyl)rhamnoside were the most abundant flavonols. Cyanidin-3-*O*-galactoside was found to be the main anthocyanin in lingonberries.

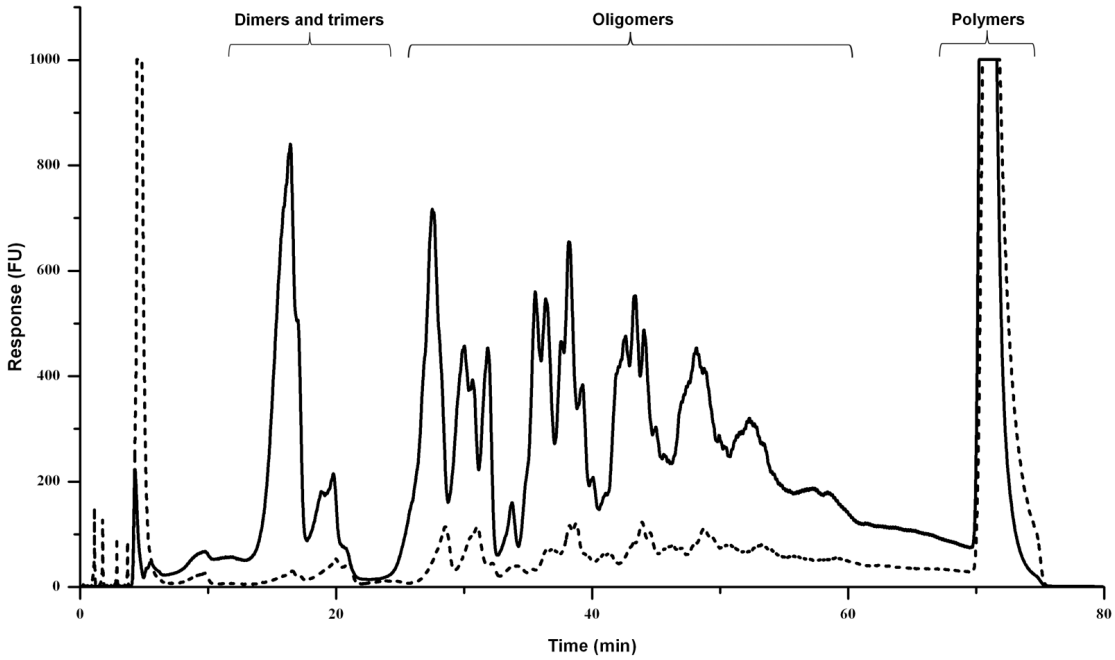


Figure 8. Preparative HPLC chromatogram of cranberry [----] and lingonberry [—] proanthocyanidins.

Table 10. Structural composition (percent in moles) of lingonberry and cranberry proanthocyanidin fractions after thioacidolysis. mDP, mean degree of polymerization.

Fraction	Terminal unit				Extention unit	mDP
	Catechin (%)	Epicatechin (%)	A-type dimer (%)	A-type trimer (%)	(%)	
Lingonberry						
1	19.2	3.4	20.3	-	57.2	2.3
2	14.8	2.3	7.9	0.5	74.5	3.9
3	12.5	1.9	5.4	-	80.2	5.0
4	10.5	1.4	5.0	-	83.1	5.9
5	9.3	1.1	4.6	-	85.0	6.7
6	10.4	1.4	3.3	0.3	84.6	6.5
7	8.7	0.8	2.9	-	87.6	8.1
8	7.5	1.1	3.3	-	88.2	8.4
9	7.6	0.8	1.9	-	89.7	9.8
10	2.5	0.5	0.1	-	96.9	32.0
Cranberry						
1	21.6	12.1	0.8	7.9	57.6	2.4
2	16.0	8.7	0.2	0.7	74.5	3.9
3	12.8	7.1	-	0.2	79.8	5.0
4	11.1	6.4	-	0.1	82.5	5.7
5	9.6	5.3	-	-	85.1	6.7
6	2.1	0.7	-	-	97.1	35.0

5.1.3 Cloudberries (III, V)

The phenolic profile of cloudberry extract consisted of ellagitannins (77.1%), proanthocyanidins (5.9%), hydroxybenzoic acids (3.2%), hydroxycinnamic acids (8.8%), ellagic acid (2.1%), flavonols (2.5%), and anthocyanins (0.4%). Cloudberry extract also contained 6% (w/w) fructose and glucose and trace amount of organic acids. Previously, it was reported that in cloudberries, ellagitannins comprised over 90% of the total phenolic content (Mylnikov et al. 2005; Heinonen, 2007).

Figure 9 shows the UPLC chromatogram of cloudberry extract detected by DAD at 280 nm, while **Table 11** shows the MS traces of the tentatively identified peaks. Two the most abundant ellagitannins in cloudberries were detected to be lambertianin C and sanguiin H-6 (**Figure 10**). The identification was based on the fragmentation patterns found from the previous studies (Tanaka et al. 1993a; Tanaka et al. 1993b; Hager et al. 2008; McDougall et al. 2008; Kool et al. 2010). Other abundant ellagitannins tentatively identified were pedunculagin or casuariin isomers, and sanguiin H-6 and H-10 isomers. All peaks listed gave the fraction ion at *m/z* 301 in negative mode representing ellagic acid; thus, they were identified as ellagitannins.

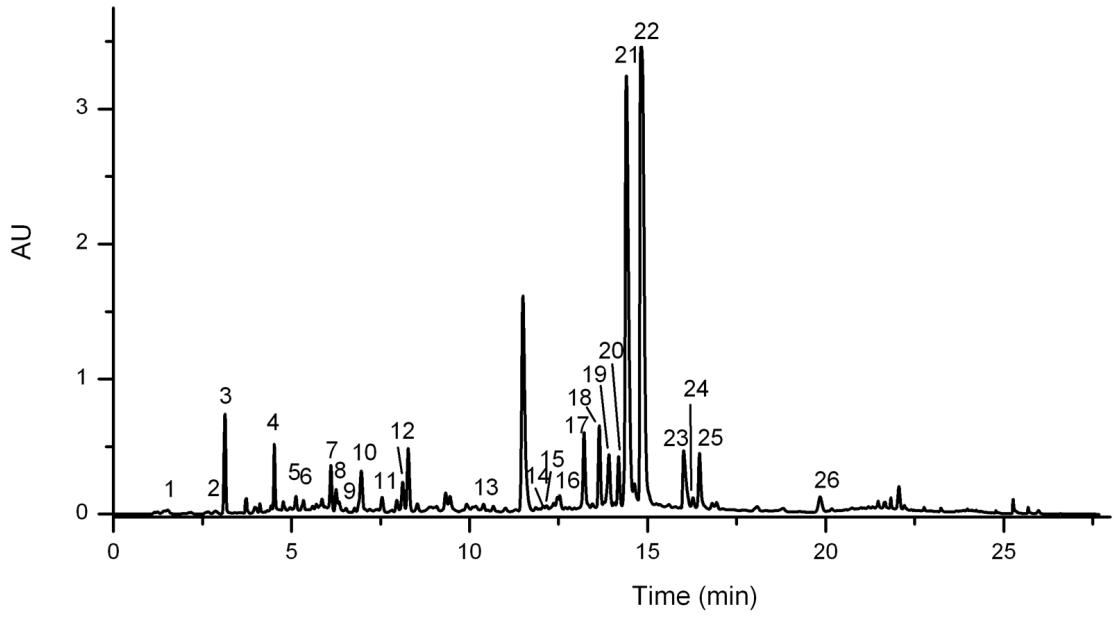


Figure 9. UPLC Chromatogram of cloudberry ellagitannin extract at 280 nm. Peaks are listed in **Table 11.**

The fragmentation patterns found from the literature were the basis for the identification. The main cleavages from the ellagitannins were the HHDP group, glucose, and the galloyl group. Peaks 4, 7, and 11 were identified as pedunculagin or casuariin (Hager et al. 2008; Moilanen and Salminen 2008; Boulekbache-Makhlouf et al. 2010). Peaks 4 and 7 exhibited at *m/z* 1567 ($[2M-H]^-$) and peak 11 at *m/z* 783. The MS^2 fragments of peaks 4 and 7 gave ions at *m/z* 783. The further fragmentation of the ions of 783 gave fragments at *m/z* 481 and 301. Peaks 24 and 26 were recognized as sanguiin H-2 (Tanaka et al. 1993a; Kool et al. 2010). Peaks 16, 18, and 19 were identified as sanguiin H-10 isomers based on the fragmentations suggested by Kool et al. (2010).

Peak 21 fragmented as lambertianin C and showed the double-charged ion at *m/z* 1401 (Hager et al. 2008; McDougall et al. 2008). Peak 22 at *m/z* 1869 was found to be sanguiin H-6, as Hager et al. (2008), McDougall et al. (2008), and Kool et al. (2010) have suggested. Peak 23 was ellagic acid because of its characteristic fragmentation. Peaks 14 and 15 at *m/z* 935 were identified as potentillin or casuarictin based on the previously published data (Salminen et al. 2001). Several small peaks with the *m/z* at 951 were recognized as trisgalloyl HHDP glucose isomers (Boulekbache-Makhlouf et al. 2010). The $[M-H]^-$ of the peaks 1 and 3 at *m/z* 969 fragmented similarly to what Zhang et al. (2009) described. Peaks 12 and 20 remain unknown, but they exhibited similarities in their fragmentations. Both of them gave fragments at *m/z* 1235, 933, and 633, which is characteristic of ellagitannins.

In blackberries and raspberries, the main ellagitannins detected were sanguiin H-6, casuarictin, potentillin, and pedunculagin (Clifford and Scalbert 2000; Hager et al. 2008). Kool et al. (2010) reported that another *Rubus* berry, the boysenberry, contained sanguiin H-6 and sanguiin H-10 as the most abundant ellagitannins. They also noted that sanguiin H-2 was found as a minor fraction. McDougall et al. (2008) analyzed cloudberries and raspberries, and they found that lambertianin C predominated in cloudberries and sanguiin H-6 in raspberries. Other abundant phenolic compounds in cloudberries according to McDougall et al. (2008) were sanguiin H-6, sanguiin H-10, and ellagic acid. In our study, we obtained similar results to those of McDougall et al. (2008); the main ellagitannins were lambertianin C and sanguiin H-6. Other ellagitannins found previously, such as sanguiin H-2 and sanguiin H-10, were also detected in our investigations.

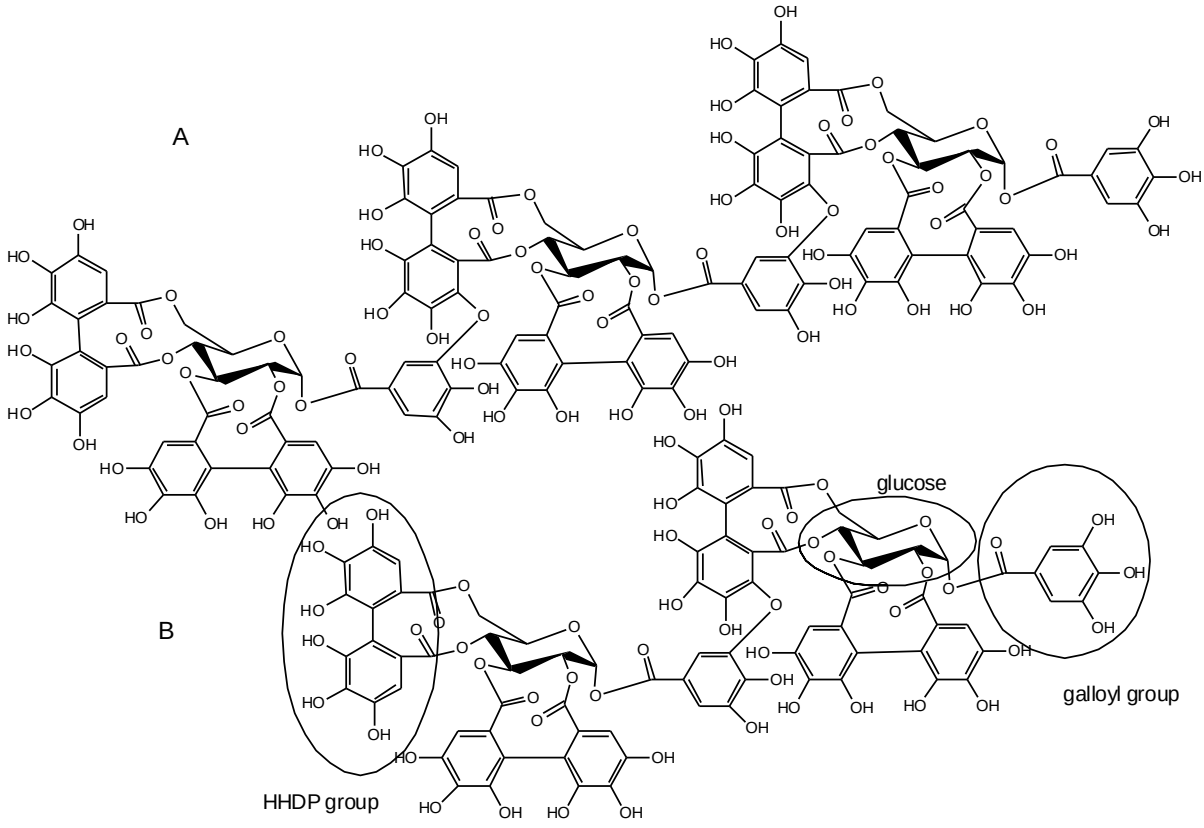


Figure 10. Structures of the main ellagitannins in cloudberries. A: lambertianin C, B: sanguiin H-6.

Table 11. Chromatographic and mass spectral features of ellagitannins in cloudberry after UPLC-MS^2 detection.

Peak	RT (min)	Tentative identity	*m/z* $[M-H]^-$	MS^2 Fragment ions *m/z*
1	1.5	phyllanthusiin G	969	925 ($[M-H]^-$ - CO_2, 481 ($[HHDP-glu]^-$), 301
2	2.8	trisgalloyl HHDP glucose isomer	951	907 ($[M-H]^-$ - CO_2), 783 (bis-HHDP-glu), 469 ($[M-H]^-$ - HHDP-glu) , 301
3	3.6	phyllanthusiin G	969	925 ($[M-H]^-$ - CO_2, 481 ($[HHDP-glu]^-$), 301
4	4.5	pedunculagin / casuariin	1567 ($[2M-H]^-$)	783 ($[M-H]^-$), 481 ($[M-H]^-$ - HHDP), 301
5	5.1	trisgalloyl HHDP glucose + HHDP glucose	1433	951 ($[M-H]^-$ - HHDP-glu), 907 ($[M-H]^-$ - HHDP-glu - CO_2), 469 ($[M-H]^-$ - HHDP-glu - CO_2 - HHDP-glu), 301
6	5.3	trisgalloyl HHDP glucose isomer	951	907 ($[M-H]^-$ - CO_2), 783 (bis-HHDP-glu), 469 ($[M-H]^-$ - HHDP-glu), 301
7	6.1	pedunculagin / casuariin	1567 ($[2M-H]^-$)	783 ($[M-H]^-$), 481 ($[M-H]^-$ - HHDP), 301
8	6.3	trisgalloyl HHDP glucose isomer	951	907 ($[M-H]^-$ - CO_2), 783 (bis-HHDP-glu), 469 ($[M-H]^-$ - HHDP-glu), 301
9	6.5	trisgalloyl HHDP glucose isomer	951	907 ($[M-H]^-$ - CO_2), 783 (bis-HHDP-glu), 469 ($[M-H]^-$ - HHDP-glu), 301
10	7	trisgalloyl HHDP glucose + galloyl	1121	1077 ($[M-H]^-$ - CO_2), 951 ($[M-H]^-$ - galloyl), 783 (bis-HHDP-glu), 301
11	7.3	pedunculagin / casuariin	783	481 ($[M-H]^-$ - HHDP), 301
12	8.1	ellagitannin	1567	1235, 933 (1235 – HHDP), 783, 301
13	10.4	trisgalloyl HHDP glucose isomer	951	907 ($[M-H]^-$ - CO_2), 783 (bis-HHDP-glu), 469 ($[M-H]^-$ - HHDP-glu) , 301
14	11.8	casuarictin / potentillin	935	633 ($[M-H]^-$ - HHDP), 301
15	12.2	casuarictin / potentillin	935	633 ($[M-H]^-$ - HHDP), 301
16	12.5	sanguiin H-10	1567	1265 ($[M-H]^-$ - HHDP), 1103 ($[M-H]^-$ - HHDP-glu), 933 ($[M-H]^-$ - HHDP-glu-galloyl), 301
17	13.2	sanguiin H-6	1869	1567 ($[M-H]^-$ - HHDP), 1265 ($[M-H]^-$ - HHDP-HHDP), 1103 ($[M-H]^-$ - HHDP-HHDP-glu), 933 ($[M-H]^-$ - HHDP-HHDP-glu-galloyl), 631 ([M-H]- - HHDP-HHDP-glu-galloyl-HHDP), 301
18	13.7	sanguiin H-10	1567	1265 ($[M-H]^-$ - HHDP), 1103 ($[M-H]^-$ - HHDP-glu), 933 ($[M-H]^-$ - HHDP-glu-galloyl), 301
19	13.9	sanguiin H-10	1567	1265 ($[M-H]^-$ - HHDP), 1103 ($[M-H]^-$ - HHDP-glu), 933 ($[M-H]^-$ - HHDP-glu-galloyl), 301
20	14.1	ellagitannin	1251	1235, 1099, 933 (1235 – HHDP), 633 (HHDP-glu-galloyl), 301

HHDP: hexahydroxydiphenoyl, glu: glucose

Table 11. Continued

Peak	RT (min)	Tentative identity	m/z $[M-H]^-$	MS^2 Fragment ions m/z
21	14.4	lambertianin C	1401 $(M-2H]^{2-})$	1869 ($[M-H]^-$ - galloyl-bis-HHDP-glu), 1567 ($[M-H]^-$ - galloyl-bis-HHDP-glu-HHDP), 1265 ([M-H]- - galloyl-bis-HHDP-glu-HHDP-HHDP), 1103 ([M-H]- - galloyl-bis-HHDP-glu-HHDP-HHDP-glu), 933 ([M-H]- - galloyl-bis-HHDP-glu-HHDP-HHDP-glu-galloyl), 631 ([M-H]- - galloyl-bis-HHDP-glu-HHDP-HHDP-glu-galloyl-HHDP), 301
22	14.8	sanguiin H-6	1869	1567 ($[M-H]^-$ - HHDP), 1265 ($[M-H]^-$ - HHDP-HHDP), 1103 ($[M-H]^-$ - HHDP-HHDP-glu), 933 ([M-H]- - HHDP-HHDP-glu-galloyl), 631 ([M-H]- - HHDP-HHDP-glu-galloyl-HHDP), 301
23	16.1	ellagic acid	301	257, 229
24	16.3	sanguiin H-2	1103	1059 ($[M-H]^-$ - CO_2), 933 ($[M-H]^-$ - galloyl, 633 (HHDP-glu-galloyl)
25	16.4	galloyl-bis-HHDP glucose isomer	935	633 ($[M-H]^-$ - HHDP), 301
26	19.9	sanguiin H-2	1103	1059 ($[M-H]^-$ - CO_2), 933 ($[M-H]^-$ - galloyl, 633 (HHDP-glu-galloyl)

HHDP: hexahydroxydiphenoyl, glu: glucose

5.2 Encapsulation and storage stability of cloudberry phenolics (V)

Maltodextrins are obtained from starch after hydrolysis, and are used as a carrier material for food powders (Estupiñan et al. 2011). By microencapsulation, the stability and shelf life of the phenolic extracts can be improved against oxygen, light, moisture, and other conditions (Robert et al. 2010). At the beginning of the storage, both microcapsules MC 5-8 and MC 18.5 and unencapsulated extract had similar phenolic profiles with ellagitannins (ET), proanthocyanidins, and hydroxycinnamic acids (OH-C) as their main phenolics (**Figure 11**). During the storage period at different relative vapor pressures (RVP), the total contents of phenolic compounds decreased; the more humid conditions, the more the total content decreased. When stored 64 days at 0, 33, and 66% RVP, the most significant changes in the phenolic profile of unencapsulated extract and microencapsules occurred in proportion to ellagitannins and ellagic acids; the content of ellagitannins decreased and ellagic acids increased.

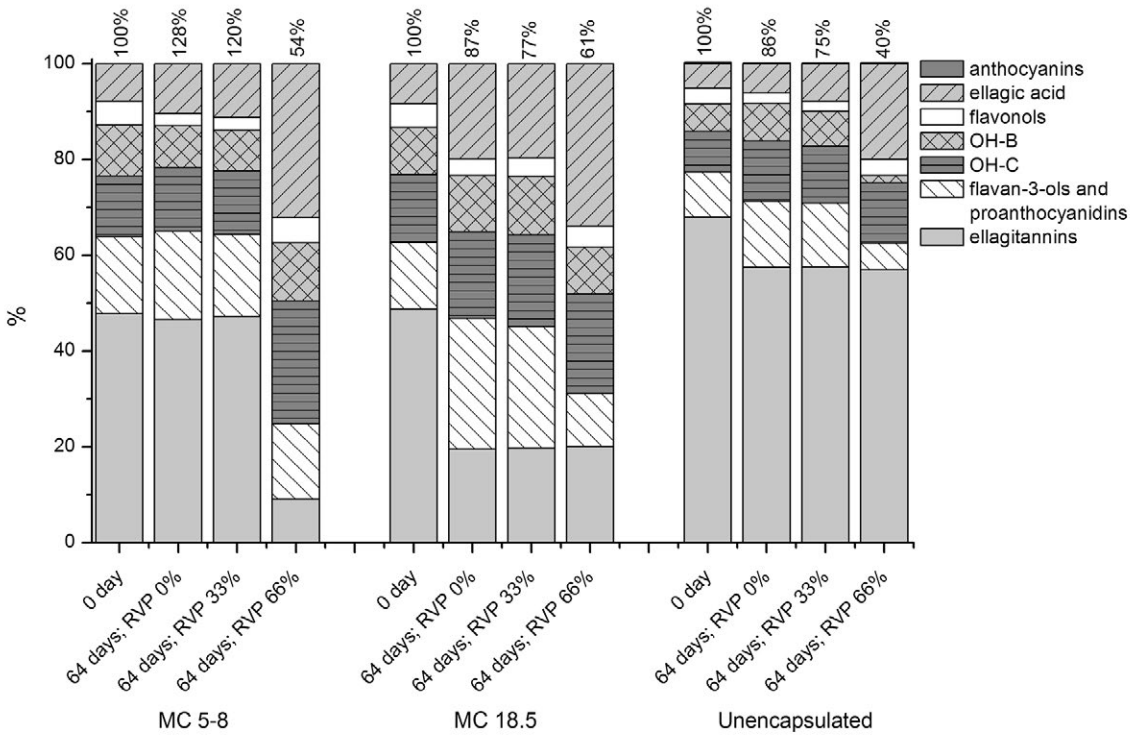


Figure 11. Phenolic profiles of cloudberry microencapsules (MC 5–8 and MC 18.5) and extract after the storage for 64 days at different conditions. Phenolic contents are expressed as percentages of phenolics remaining. OH-B, hydroxybenzoic acids; OH-C, hydroxycinnamic acids.

In contrast to the unencapsulated extract and microcapsule MC 18.5, phenolic profiles of microcapsule MC 5−8 remained practically unchanged at 0 and 33% RVP, further adding to the conclusion that microencapsulation with maltodextrin MD5−8 improved the storage stability of cloudberry phenolics.

Unencapsulated ellagitannins were sensitive to storage under all conditions tested (**Figure 11**). At 66% RVP, neither unencapsulated extract nor microcapsules managed to avoid changes in phenolic profiles. The phenolic classes in unencapsulated extract after storage at 66% RVP for 64 days were ellagitannins (57%), ellagic acid (20%), and hydroxycinnamic acids (13%), while ellagic acid (32%), hydroxycinnamic acids (26%), and proanthocyanidins (16%) were the predominant classes in MC 5−8 and ellagic acid (34%), hydroxycinnamic acids (21%), and ellagitannins (20%) in MC 18.5.

In the unencapsulated cloudberry extract, a statistically significant decrease in the content of ellagitannins was observed after 64 days of storage at 0 and 33% RVP, but at 66% RVP, the degradation of ellagitannins was already significant after 32 days. At the end of the storage period, only 73% (at 0% RVP), 63% (at 33% RVP), and 34% (at 66% RVP) of the original amount of ellagitannins remained in the powder. In contrast, the content of ellagic acids was higher in relation to greater storage RVP. After 64 days, ellagic acid contents were 104% (at 0% RVP), 116% (at 33% RVP), and 159% (at 66% RVP) of the original ellagic acid content.

In MC5-8 capsules, phenolic profiles were stable at 0 and 33% RVP for 64 days, while significant changes were seen at 66% RVP. In MC 18.5 capsules the phenolic profiles were altered notably even at 0% RVP in comparison to day 0. Encapsulation with either type of maltodextrin resulted in decreased formation of ellagic acids during 32 days of storage. However, after 32 days of storage, the content of ellagic acid in microencapsules increased rapidly by more than twofold at 33% RVP and more than threefold at 66% RVP. The content of ellagic acids was lower in MC 5−8 than MC 18.5. The proportion of ellagic acids on the surface of MC 5−8 remained unchanged during the whole storage period, whereas in the MC 18.5, the contents of surface ellagic acids increased after 32 days.

Proanthocyanidins in unencapsulated cloudberry extract remained unaltered at 0 and 33% RVP. At 66 % RVP, the content of proanthocyanidins decreased significantly during the whole storage period, and only 24% of the initial proanthocyanidins were left after 64 days of storage. The content of hydroxycinnamic acids in unencapsulated extract remained constant or even increased slightly at low RVP. Hydroxybenzoic acids (OH-B) were stable under dry storage conditions. MC 5−8 improved the stability of flavonols at 0% RVP up to 32 days and at 33% RVP during the whole storage period, while the stability could not be enhanced at 66% RVP. MC 18.5 was unable to protect flavonols at any RVP. This instability of proanthocyanidins has also been found in other studies, e.g., the concentration of proanthocyanidins in strawberry purees decreased when the purees were stored at 22 °C for 8–16 weeks (Aaby et al. 2007) and the content of monomeric proanthocyanidin, catechins, in liquid blueberry extract decreased remarkably during storage at 23 °C for 60 days (Srivastava et al. 2007). Microencapsulation did not improve the stability of proanthocyanidins at 66% RVP, even though the loss was not statistically significant in MC 18.5.

The losses of ellagitannins in cloudberry extract can probably be attributed to the hydrolysis of ellagitannins, which liberates ellagic acid. The hydrolysis of ellagitannins has been suggested in previous studies to occur during various processes and storage. Aaby et al. (2007) observed that strawberry puree made from flesh retained all ellagitannins during 16 weeks of storage at 22 °C. In contrast, purees made from strawberry homogenate and achene-enriched fraction lost

20−28% of their initial ellagitannins, and at the same time, the concentration of ellagic acid almost doubled; this was explained by the hydrolysis of ellagitannins. In another study, the free ellagic acid content of raspberries increased 2.5-fold during jam processing (Zafrilla et al. 2001). The authors speculated that cooking released ellagic acid from the ellagitannin structure, or alternatively, that ellagic acid could be more easily extracted from the processed product than from berries as such. Lei et al. (2001) found that the main ellagitannins of oak wood degraded in aqueous solutions even at room temperature in the absence of oxygen. Hydrolysis was thought to be one of the changes oak ellagitannins underwent since the results from the HPLC analyses of the degradation reactions indicated formation of ellagic acid. In the processed products of ellagitannin-rich blackberries, only minimal changes in the total ellagitannin content were observed during the storage, but compositional changes were detected (Hager et al. 2010). A possible explanation was that the larger molecular weight ellagitannins, such as lambertianin C, sanguiin H-6, or sanguiin H-10, were depolymerized to smaller ellagitannins or ellagic acid.

All the above-mentioned ellagitannin losses occurred in moist conditions (purees, jams, aqueous solutions). In our study, the decrease in the ellagitannin content occurred also under low-RVP conditions. Thus, the losses of ellagitannins could not be explained only by a hydrolysis reaction, but also by oxidation. Oxidation likely explains the results obtained by Salminen (2003). He observed that in freeze-dried and powdered birch leaves, the concentration of the main ellagitannin (pedunculagin derivate) decreased during one-year dry storage in sealed plastic bags at 22 °C in the dark. In the presence of oxygen or other radicals (such as iron that might be presented as trace amounts in maltodextrins), ellagitannin may polymerize into oligomeric and polymeric structures via a radical-mediated oxidation pathway (Shahidi and Naczk 2003; Nohynek et al. 2006). This reaction is analogous to the autoxidation of polyunsaturated lipids (Shahidi and Naczk 2003). The oxidation of phenolics may result in the formation of quinones and other yet unknown products. In our study, the disappearance of ellagitannins may be partly explained by ellagitannin oligomers and polymers, while only ellagitannins up to trimeric structures could be analyzed with the HPLC method used.

Encapsulation was advantageous for the storage stability of ellagitannins. However, the stability of encapsulated ellagitannins was strongly dependent on storage RVP and the capsule material used. MD 5−8 proved to be a better capsule material than MC 18.5.

5.3 Radical scavenging and antioxidant activity

5.3.1 Radical scavenging and antioxidant activity of hydroxycinnamic acid glycosides (IV)

DPPH$^{\bullet}$ radical scavenging assay

The radical scavenging activity of the hydroxycinnamic acid glycoside esters was determined at a concentration of 1 mg/mL based on the total phenolic content in DPPH$^{\bullet}$ assay (Figure 12). Among the tested compounds, 6-O-sinapoyl-glucoside had the best radical scavenging activity (96%). The other sinapic acid derivatives were active as well, specifically free sinapic acid (91%), the mixture of sinapoyl glucosides (91%) and 2-O-sinapoyl-glucoside (91%).

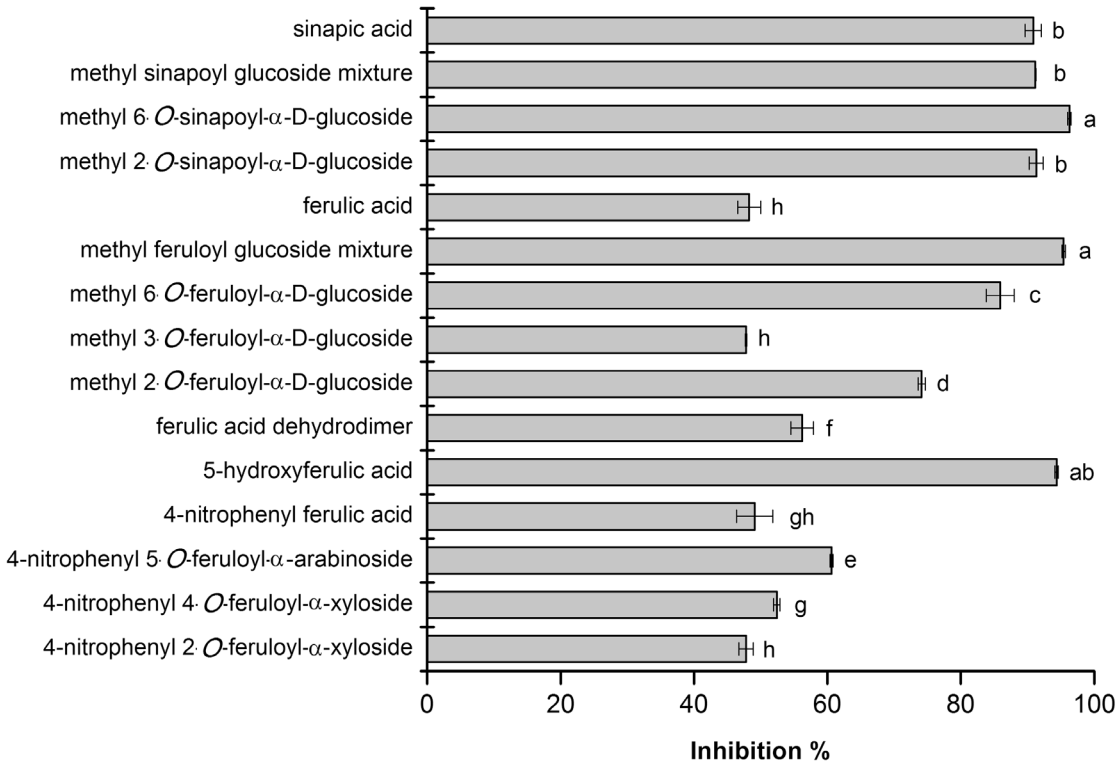


Figure 12. DPPH• radical scavenging activity of hydroxycinnamic glycoside esters.

The mixture of different feruloyl glucosides was more effective in the radical scavenging test than free ferulic acid and individual isomers of glucoside esters (**Figure 12**). The mixture, which mainly contained 6-*O*- and 2-*O*-feruloyl-glucosides, exhibited a scavenging activity of 95%. Comparing the different isomers of feruloyl glucosides, the esterification to the primary C6 hydroxyl group resulted in significantly ($p < 0.05$) more effective radical scavenging (86%) than esterification to the secondary C2 or C3 positions.

In the DPPH• test, the number of hydroxyl groups is significant in assessing radical scavenging activity. Therefore, no difference between the feruloyl glucosides was anticipated, which is contrary to the findings. A possible explanation for the lower radical scavenging activity toward the DPPH radical when using 2-*O*- and 3-*O*-feruloyl glucosides is that there was a slight difference in the solubility in the test solvent, methanol. The presence of the –CH=CH–COOH chain in hydroxycinnamic acids ensures hydrogen-donating ability and subsequent radical stabilization (Zhang et al. 2006).

Ferulic acid esterified to the arabinofuranosyl moiety was the most effective, its scavenging activity being 61%, which might be due to the slight solubility differences compared to xylopyranoside forms. There was a slight difference compared to the feruloyl group linked to position C4 (52%) and C2 (48%) of xylopyranoside, or directly to 4-nitrophenol (49%). Overall, sinapic acid or ferulic acid esterified to the primary hydroxyl of glycoside was a better radical scavenger to the secondary hydroxyl groups than esters.

These results, as well as previous studies on hydroxycinnamic acids (Pekkarinen et al. 1999), suggest that the naturally existing glycoside esters are at least as potent in preventing lipid oxidation as free phenolic acids. More hydrophilic glucoside esters of hydroxycinnamic acids are more active radical scavengers compared with the free forms of acids. These findings contradict earlier reports. Tominaga et al. (2005) found that the introduction of the β-glucose moiety to caffeic and ferulic acids decreased their radical scavenging activity due to increased hydrophilicity and permeability in aqueous solutions in DPPH tests. According to Cos et al. (2002), caffeic acid had the highest scavenging activity, followed by ferulic and sinapic acids, while the esterification of caffeic acid with quinic acid decreased the activity.

Sinapic acid derivatives of the hydroxycinnamic acid glycosides were the most potent in scavenging the DPPH$^{\bullet}$ radicals. The esterification of glucose to the primary (C6) hydroxyl group enhanced the radical scavenging activity of ferulic acid.

Liposome and emulsion

The antioxidant activity was measured in the liposome model system at 4.2 μg/mL and 8.4 μg/mL concentrations based on the total phenolic content. **Figure 13** shows the antioxidant activities of sinapic acid and ferulic acid glucosides at a concentration of 4.2 μg/mL. The esterification of sinapic acid had no effect on the inhibition of hexanal formation. Sinapic acid, 2-*O*-sinapoyl glucoside, 6-*O*-sinapoyl glucoside, and sinapoyl glucoside mixture were all as effective. Sinapic acid has earlier been shown to be a more effective antioxidant than ferulic acid, which in turn is more effective than *p*-coumaric acid (Cos et al. 2002). Ferulic acid maintained the activity toward hexanal formation if the esterification occurred at the C6-position. 2-*O*-feruloyl and 3-*O*-feruloyl glucosides were significantly less effective than ferulic acid. In addition, the mixture of feruloyl glucosides was not as effective as ferulic acid or 6-*O*-feryloyl glucoside, probably due to the lower activity of 2-*O*-feruloyl and 3-*O*-feruloyl glucosides. Nyström et al. (2005) have demonstrated that bulk and emulsified methyl linoleate steryl ferulates had similar activity against lipid oxidation than ferulic acid had. Ferulic acid, sinapic acid, 2-*O*-sinapoyl, 6-*O*-sinapoyl, and 6-*O*-feruloyl had similar effectiveness in inhibiting liposome oxidation, while ferulic acid glucosides were less powerful. Andreasen et al. (2001) tested the inhibition of LDL oxidation in copper induced assay by hydroxycinnamic acids and ferulic acid dehydrodimers. The results revealed that caffeic acid was the most potent inhibitor against LDL oxidation, followed by sinapic acid, ferulic acid dehydrodimer (8-*O*-4-diFA), ferulic acid, and *p*-coumaric acid. Feruloylated arabinoxylotrisaccharides from wheat enhanced the activity toward LDL oxidation, while the activity was reduced compared to ferulic acid in the DPPH test (Katapodis et al. 2003; Yuan et al. 2005). Antioxidants trapped near the surface of the liposomal membrane are better antioxidants, as shown with hexyl and octyl ferulates, which are more effective than shorter alkyl esters (Materska and Perucka 2005; Tominaga et al. 2005). Monomeric sinapic acid showed the antioxidant activity toward LDL oxidation followed by ferulic acid and *p*-coumaric acid, but dehydrodimers were more effective in the inhibition of LDL oxidation. Non-cyclized dehydrodimers were also found to act as radical scavengers, while cyclized forms of dehydrodimers were active in chelating copper ions (Neudörffer et al. 2006).

4-Nitrophenyl 5-*O*-feruloyl-arabinoside was the most powerful antioxidant among the 4-nitrophenyl feruloyl glycosides. The inhibitions of 4-nitrophenyl ferulate, 4-nitrophenyl 4-*O*-feruloyl-xyloside, and 4-nitrophenyl 5-*O*-feruloyl-arabinoside against hexanal formation varied between 85 and 93%

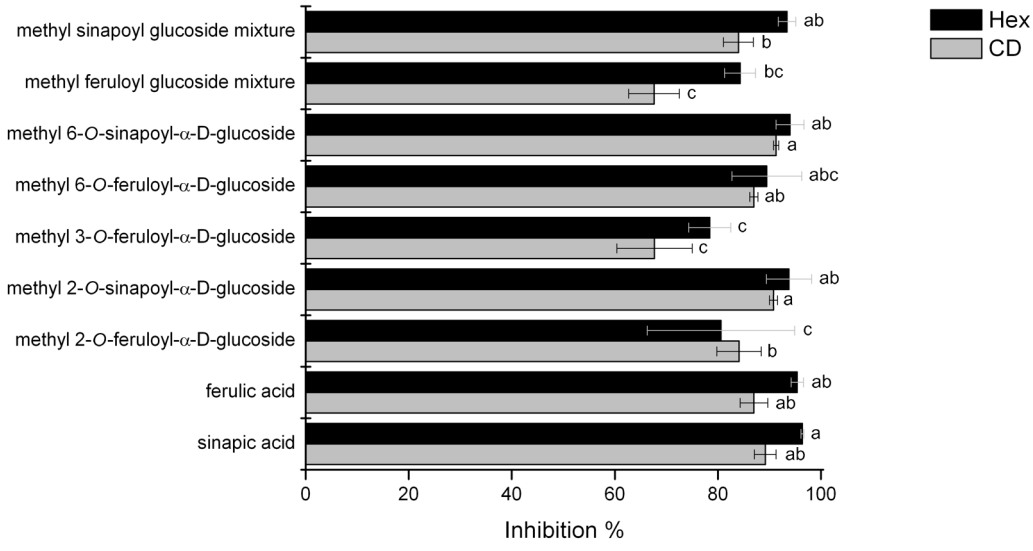


Figure 13. Antioxidant activity of sinapic acid and ferulic acid glucosides in a liposome oxidation model at 4.2 µg/mL concentration. CD, conjugated diene hydroperoxides; Hex, hexanal.

Hydroxycinnamic acid glycosides were tested at 50 and 100 µg/g concentrations in an emulsion oxidation model. At 50 µg/g concentration, ferulic acid and sinapic acid equally inhibited the hexanal formation (**Figure 14**). The esterification of ferulic acid to the C6 position in glucose slightly enhanced the antioxidant activity, while esterification to the C2 or C3 positions in methyl 2-*O*-feruloyl-α-D-glucoside and methyl 3-*O*-feruloyl-α-D-glucoside did not. C2 and C3 esters were significantly less effective than C6 ester. On the other hand, esterification of sinapic acid to glucose moiety at different positions increased their ability to inhibit the hexanal formation. Compared to the sinapic acid, sinapic acid esters were slightly more effective. Both sinapic acid and ferulic acid glucoside mixtures prevented the hexanal formation as effectively as 2-*O*-sinapoyl, 6-*O*-sinapoyl, and 6-*O*-feruloyl glucosides. Feruloyl-β-D-glucopyranosides, sinapoyl-β-D-glucopyranosides, and caffeoyl-β-D-glucopyranosides were reported as being weaker antioxidants than free corresponding phenolic acids toward the inhibition of oxidation of β-carotene-linoleic acid emulsions (Materska and Perucka 2005; Tominaga et al. 2005). In our study, feruloylglucosides were slightly weaker than free ferulic acid in inhibiting the lipid oxidation in emulsion, while sinapic acid and its derivatives were equally active. In conclusion, hydroxycinnamic acid esters were as potent antioxidants as free acids, as well as being effective in the oxidation of liposomes and emulsions. In general, 6-*O*-feruloyl-glucoside exhibited the highest antioxidant activity, followed by ferulic acid, 2-*O*-feruloyl-glucoside, and 3-*O*-feruloyl-glucoside.

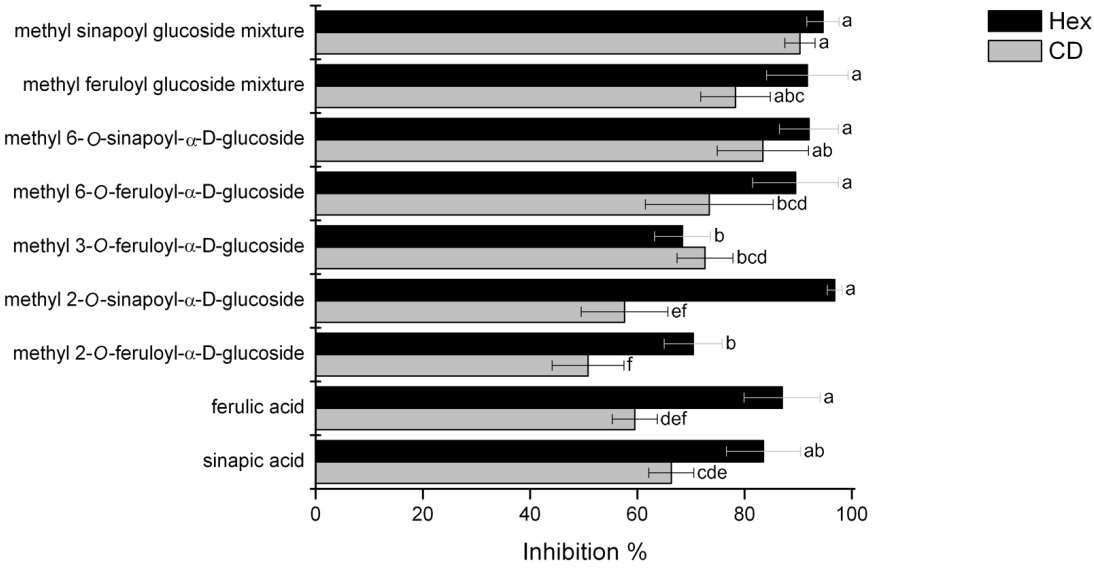


Figure 14. Antioxidant activity of sinapic acid and ferulic acid glucosides in the emulsion oxidation model at 50 µg/g concentration. CD, conjugated diene hydroperoxides; Hex, hexanal.

In emulsions, the lipophilic antioxidants of low hydrophilic-lipophilic balance are favored (Frankel et al. 1994). The lipophilic antioxidants are adequately surface active to be located to the oil-water interface, where they are able to inhibit lipid oxidation; hydrophilic antioxidants, on the other hand, are located in the aqueous phase, and are consequently less effective against lipid oxidation (Huang et al. 1997). Hydroxycinnamic acids are noticed to act as chain-breaking antioxidants and reducing agents (Shahidi and Chandrasekara 2010). The oil-water interface appears to be significant for antioxidants to function as chain breakers. Emulsifiers, which are amphiphilic, are located in the oil-water interfaces in emulsions and can solubilize antioxidants, thus enabling polar antioxidants to get in contact with lipids. Stöckmann et al. (2000) showed that emulsions containing partially hydrolyzed soybean lecithin as emulsifier solubilized gallic acid more efficiently than its more hydrophobic derivative ethyl gallate.

Feruloyl and sinapoyl glucosides are more hydrophilic than their free forms. Due to the ability of soybean lecithin to solubilize hydrophilic compounds, the tested glucosides were shown to be effective antioxidants. Altering the pH changes the solubility of hydroxycinnamic acids to the aqueous phase, and lowering the pH might be related to the dissociation of the functional group. Schwarz et al. (1996) observed that the concentrations of ferulic acid and caffeic acid decreased in the aqueous phase of water/oil systems when the pH was lowered from 7.0 to 3.0. Introduction of sugar to the phenolic compounds would make them more hydrophilic and inhibit them from reaching the oil-water interface (Zhou et al. 2005). These findings were confirmed in the present study, where the tested hydrophilic antioxidants were not as effective in emulsions as in liposomes. The pH in the present study was 5.0 in the liposomes and neutral in the emulsion.

In emulsion and liposome oxidation, ferulic acid and its derivatives had similar antioxidant activity indicating that the introduction of sugar moiety to the hydroxycinnamic acid does not reduce the capability for the antiradical potential. The antioxidant activity of phenolic compounds in liposomes depends on both the location and orientation of the antioxidant in the system. Lipophilic antioxidants can act both by scavenging of aqueous peroxyl radicals, and by scavenging of lipid peroxyl radicals within the liposomal membrane. The more hydrophilic 5-hydroxy ferulic acid has a lower hydrophilic-lipophilic balance, and therefore it was more a powerful antioxidant compared with ferulic acid and the ferulic acid dehydrodimer. In liposomes, the order was reversed, with ferulic acid dehydrodimer being the most effective. In acidic conditions, phenolic acids are weak antioxidants, while in increasing their pH, their antioxidant activity increases (Amorati et al. 2006). The explanation for the better antioxidant activity in basic conditions is that there is a rapid electron transfer from anionic phenolic acids to peroxyl radicals. Hydrogen abstraction and electron transfer in the antioxidant reactions depend on the conditions, such as pH value and the stability of the intermediate radicals (Zhou et al. 2005).

5.3.2 Antioxidant activity of rowanberries, cranberries, lingonberries, and cloudberries (I-II,V)

Rowanberries

In the liposome oxidation model, rowanberry phenolics were the most effective antioxidants at all concentrations tested compared to cloudberries, cranberries, and lingonberries. The inhibition of hexanal formation, a secondary lipid oxidation product, was over 90% at the concentrations of 4.2 or 8.4 μg/mL of rowanberry phenolics (**Table 12**). Indeed, the inhibitory effect on lipid oxidation remained high (86–90%) at lower amounts (2.1 μg/mL) of rowanberry phenolics. Rowanberry phenolics inhibited the conjugated diene hydroperoxide formation the most effectively at concentrations of 2.1 and 4.2 μg/mL, while lingonberry and cranberry oligomeric and polymeric proanthocyanidins were less active. The lingonberry phenolic extract and cranberry dimeric and trimeric fraction were as effective as those of rowanberries. At 8.4 μg/mL, rowanberry phenolics, as well as lingonberry and cranberry dimers and trimers, were the most efficient. The antioxidant activity of cloudberry ellagitannins was of the same order of magnitude as cranberry oligomeric and polymeric proanthocyanidins. The trend in the inhibition of hexanal formation was the same as the inhibition of conjugated diene hydroperoxides; rowanberry phenolics were the most active. Lingonberry oligomeric proanthocyanidins and phenolic extract, cranberry dimers and trimers, and cranberry phenolics were equal antioxidants to rowanberries at a concentration of 2.1 μg/mL.

Compared to our previous studies, where similar oxidation models at the same concentration levels of berry phenolics were used, the antioxidant activity of rowanberry phenolics was excellent. The inhibition varied between 74% and 80% for conjugated diene hydroperoxides, and 93 and 97% for hexanal in liposomes; the previous results for various berries varied between 15 and 70% for conjugated diene hydroperoxides and 41 and 99% for hexanal (Viljanen et al. 2004a; Vuorela et al. 2005). In liposomes and emulsions, oxidation was inhibited in a dose-dependent manner regarding the formation of primary lipid oxidation products, specifically

conjugated diene hydroperoxides, although the increase in inhibition accompanying the transition from 50 to 100 µg/g phenolic compound was not statistically significant.

Table 12. Antioxidant activity of rowanberry, lingonberry, cranberry, and cloudberry phenolics in liposome and emulsion oxidation models (Inhibition % ± SD).

Liposome						
	Conjugated dienes (%)			Hexanal (%)		
	2.1 µg/mL	4.2 µg/mL	8.4 µg/mL	2.1 µg/mL	4.2 µg/mL	8.4 µg/mL
Wild rowanberry	67.5 ± 2.2^{a}	76.9 ± 2.0^{a}	77.4 ± 0.5^{ab}	90.3 ± 1.5^{a}	95.7 ± 2.6^{ab}	96.8 ± 0.1^{abc}
Cultivated rowanberries						
Burka	63.0 ± 1.0^{ab}	74.8 ± 1.3^{ab}	79.2 ± 0.9^{a}	85.9 ± 0.9^{ab}	96.9 ± 2.1^{a}	92.7 ± 1.2^{ef}
Granatnaja	66.3 ± 0.5^{a}	75.4 ± 3.2^{ab}	77.5 ± 2.4^{ab}	87.9 ± 0.7^{a}	96.6 ± 2.7^{a}	96.5 ± 0.9^{abc}
Titan	63.8 ± 4.2^{ab}	73.9 ± 2.8^{abc}	79.8 ± 0.2^{a}	86.3 ± 2.3^{ab}	96.2 ± 2.8^{ab}	97.2 ± 0.1^{ab}
Zoltaja	68.4 ± 1.2^{a}	73.6 ± 5.9^{abc}	77.5 ± 0.3^{ab}	90.4 ± 0.6^{a}	97.0 ± 1.0^{a}	97.1 ± 0.1^{ab}
Lingonberry						
dimers and trimers	50.6 ± 4.0^{bc}	65.3 ± 0.8^{de}	77.9 ± 0.1^{ab}	76.1 ± 0.6^{bcd}	88.0 ± 1.0^{cd}	96.3 ± 0.5^{abcd}
oligomers	58.2 ± 5.0^{abc}	66.3 ± 4.2^{cde}	72.6 ± 2.6^{c}	84.8 ± 4.4^{abc}	88.9 ± 2.4^{bcd}	93.0 ± 1.0^{def}
polymers	30.8 ± 5.1^{d}	68.7 ± 4.9^{bcd}	54.1 ± 0.6^{g}	74.7 ± 5.4^{cde}	90.2 ± 7.7^{abcd}	93.6 ± 0.6^{cdef}
extract	49 ± 4.7^{bc}	73.5 ± 0.4^{abc}	74.1 ± 1.8^{bc}	83.9 ± 5.4^{abc}	92.5 ± 3.4^{abc}	94.4 ± 0.6^{bcde}
Cranberry						
dimers and trimers	47.9 ± 5.0^{c}	69.8 ± 2.2^{abcd}	80.3 ± 0.3^{a}	86.6 ± 6.5^{ab}	95.4 ± 0.3^{abc}	98.0 ± 0.1^{a}
oligomers	46.4 ± 2.3^{c}	58.8 ± 1.3^{ef}	64.8 ± 0.7^{de}	64.1 ± 4.6^{e}	79.7 ± 1.4^{e}	85.3 ± 0.8^{h}
polymers	24.6 ± 15.3^{d}	50.8 ± 1.5^{f}	60.1 ± 0.2^{g}	64.9 ± 0.8^{e}	84.5 ± 6.2^{de}	89.2 ± 1.0^{g}
extract	56.4 ± 4.7^{abc}	54.4 ± 2.9^{f}	63.0 ± 1.0^{ef}	83.2 ± 3.5^{abc}	84.4 ± 3.5^{de}	90.9 ± 2.5^{fg}
Cloudberry	nt	nt	67.9 ± 1.1^{d}	nt	nt	79.7 ± 0.9^{i}
Emulsion						
	Conjugated dienes (%)			Hexanal (%)		
	25 µg/g	50 µg/g	100 µg/g	25 µg/g	50 µg/g	100 µg/g
Wild rowanberry	79.6 ± 7.0^{a}	83.1 ± 7.2^{a}	87.4 ± 6.9^{ab}	93.9 ± 4.4^{a}	86.0 ± 11.3^{a}	95.0 ± 2.5^{ab}
Cultivated rowanberries						
Burka	73.6 ± 13.9^{a}	81.2 ± 10.9^{ab}	88.3 ± 6.9^{ab}	93.4 ± 5.2^{a}	86.5 ± 17.1^{a}	91.8 ± 6.1^{ab}
Granatnaja	79.2 ± 8.5^{a}	89.5 ± 2.5^{a}	84.7 ± 4.0^{ab}	92.7 ± 5.2^{a}	91.8 ± 6.4^{a}	96.7 ± 1.4^{ab}
Titan	76.4 ± 9.5^{a}	88.1 ± 3.3^{a}	89.7 ± 3.0^{ab}	90.3 ± 6.4^{a}	89.6 ± 10.2^{a}	88.0 ± 8.3^{b}
Zoltaja	80.6 ± 8.4^{a}	86.7 ± 1.4^{a}	83.5 ± 4.7^{b}	86.8 ± 11.5^{a}	86.3 ± 10.0^{a}	92.4 ± 2.9^{ab}
Lingonberry						
dimers and trimers	nt	90.5 ± 1.4^{a}	89.7 ± 0.5^{ab}	nt	84.9 ± 2.9^{a}	98.1 ± 0.2^{ab}
oligomers	nt	90.5 ± 0.4^{a}	89.9 ± 0.1^{ab}	nt	93.4 ± 1.2^{a}	100 ± 0.0^{a}
polymers	nt	92.6 ± 0.3^{a}	93.0 ± 0.4^{ab}	nt	98.3 ± 0.1^{a}	98.9 ± 0.1^{a}
Cranberry						
dimers and trimers	nt	69.8 ± 2.9^{b}	94.1 ± 0.5^{a}	nt	94.7 ± 1.1^{a}	93.2 ± 1.4^{ab}
oligomers	nt	26.8 ± 10.6^{c}	93.5 ± 0.1^{a}	nt	91.5 ± 5.9^{a}	98.7 ± 0.3^{a}
polymers	nt	92.6 ± 0.4^{a}	91.9 ± 0.8^{ab}	nt	99.7 ± 0.0^{a}	99.4 ± 0.2^{a}

nt, not tested

It is well accepted that the antioxidant activity of phenolic compounds such as caffeic acid and other hydroxycinnamic acids is related to the number of hydroxyl groups in their molecular structure (Chen and Ho 1997). Furthermore, it has previously been shown that the radical scavenging capacities of cultivated sweet rowanberries are high as measured with the FRAP and DPPH methods (Hukkanen et al. 2006). The antioxidant activities of wild and cultivated rowanberries were both strong and similar, even though their phenolic profiles differed. The cultivated rowanberries Titan and Burka had a higher content of anthocyanins (> 25%) and a lower content of hydroxycinnamic acids (56 and 60%), while Zoltaja contained over 75% of hydroxycinnamic acids and less than 3% of anthocyanins.

According to Heinonen et al. (1998), berries low in anthocyanins and high in hydroxycinnamates were the most potent antioxidants toward liposome oxidation. Among the anthocyanin aglycons, only malvidin appeared to be effective against liposome oxidation at 10, 20, and 40 μM concentrations, while cyanidin, delphinidin, and pelargonidin were pro-oxidants (Satue-Gracia et al. 1997). However, all these anthocyanins exhibited antioxidant activity in emulsified methyl linoleate (Kähkönen and Heinonen 2003). Pekkarinen et al. (1999) evaluated the antioxidant activity of different phenolic acids in an emulsified system, showing that at concentrations of 50 μM, caffeic acid was a pro-oxidant while at concentrations of 1000 μM, it exhibited weak antioxidant behavior. In another study, caffeic acid was found to be effective, while its derivative, chlorogenic acid, was a weaker antioxidant in a methyl linoleate emulsion model (Kähkönen and Heinonen 2003). In the present study, both wild and cultivated rowanberry phenolics rich in chlorogenic acids showed antioxidant activity toward lipid oxidation in liposome and emulsion structures. These findings suggest that antioxidant behavior is dependent on the food matrix, as was proposed earlier by Frankel and Meyer (Frankel and Meyer 2000; Heinonen 2007). The increase in anthocyanin content in the cultivated species did not significantly affect the antioxidant activity.

In the inhibition of lipid peroxidation mediated by metmyoglobin and LDL oxidation, Castelluccio et al. (1995) noticed that chlorogenic acid and caffeic acid were the most active in comparison with ferulic acid and *p*-coumaric acid. The concentrations for 50% inhibition of LDL oxidation were 0.33 μM for chlorogenic acid and caffeic acid, while the concentrations for ferulic acid and *p*-coumaric to inhibit 50% were almost 3 and 12 times higher, respectively. The higher antioxidant activity can be explained by the substituents in the molecule. The methoxy group in ferulic acid enhances its activity as an antioxidant compared to *p*-coumaric acid because of the electron-donating ability of the methoxy group. On the other hand, the methoxylation of ferulic acid decreases the antioxidant activity in contrast to caffeic acid and chlorogenic acid, in which hydroxylation occurs in place of methoxylation (Castelluccio et al. 1995).

Wild and cultivated rowanberries rich in chlorogenic acids were effective in the inhibition of lipid oxidation, and in most cases they were more active than cloudberries, cranberries, and lingonberries.

Cranberries and lingonberries

In emulsions, the inhibition of conjugated diene hydroperoxide formation at a concentration of 50 μg/g was not significantly different between lingonberry procyanidin fractions (**Table 12**). Only the polymeric fraction of cranberry proanthocyanidins inhibited the lipid oxidation as effectively as the lingonberry fractions. Phenolic compounds from cranberries and lingonberries were more effective in preventing the hexanal formation in liposome oxidation assays than conjugated diene formation, thus acting as chain-breaking antioxidants and to lesser degree inhibiting the initiation of lipid oxidation.

Differences in the antioxidant activities of emulsion oxidation were small, especially at higher (100 μg/g) concentrations. The antioxidant activity toward the hydroxyl free radical scavenging of oligomeric proanthocyanidin B3 was reported to be slightly higher than that of the two A-type proanthocyanidins (A2 epicatechin-(2β→7, 4β→8)-epicatechin and trimer epicatechin-(4β→8, 2β→O→7)-epicatechin-(4β→8)-epicatechin) from *Litchi chinensis* pericarp (Liu et al. 2007). In the present study, the oligomeric fraction of cranberry proanthocyanidins was slightly weaker than the oligomeric fraction of lingonberry proanthocyanidins. The proportion of A-type linkages was higher in lingonberry proanthocyanidin fractions than in cranberries.

Among the cranberry fractions, the dimeric and trimeric fraction of cranberry proanthocyanidins was the most effective (inhibition of formation of conjugated diene hydroperoxides and hexanal, 47.9–80.3% and 86.6–98.0%, respectively) in inhibiting the liposome oxidation at all concentrations. The oligomeric fraction of lingonberry at a concentration of 2.1 and polymeric fraction at 4.2 μg/mL were the most efficient in the inhibition of oxidation (conjugated diene hydroperoxides 58.2 and 68.7% and hexanal 84.8 and 88.9%, respectively) when comparing lingonberry fractions. The dimeric and trimeric fraction from cranberries had better antioxidant activity than the dimeric and trimeric fraction from lingonberries. The greater inhibition of conjugated diene hydroperoxide and hexanal formation may be due to the presence of A-type trimeric structures in cranberries. Ho et al. (1999) previously reported the strongest anti-lipid peroxidation activity of a proanthocyanidin trimer containing an A-type linkage. The A-type dimer also showed strong superoxide scavenging activity in their study. In liposomes at all tested concentrations, the dimeric and trimeric fraction was the most effective of the cranberry fractions. With lingonberries, different proanthocyanidin fractions were most active in liposomes at different concentrations.

In liposomes, the polymeric fractions of lingonberry and cranberry proanthocyanidins had the lowest inhibition levels at all concentrations, except for the lingonberry polymeric fraction at 4.2 μg/mL, although at higher concentration the inhibition was good. Overall, lingonberry fractions were slightly more effective than cranberry fractions in inhibiting liposome oxidation. The inhibition of hexanal formation was over 85% in almost all fractions at concentrations of 4.2 and 8.4 μg/mL. Differences between fractions were small, but in most cases, the oligomeric or polymeric fraction was the most efficient. Little information is available concerning the antioxidant activity of cranberry and lingonberry proanthocyanidins.

Viljanen et al. (2004a; 2004b) tested the commercially available procyanidins B1 (epicatechin-(4β→8)catechin) and B2 (epicatechin-(4β→8)epicatechin) dimer standards and isolated lingonberry proanthocyanidins for their antioxidant activity in lecithin liposomes. Lingonberry

proanthocyanidins were divided into two fractions: one contained mainly (92%) monomeric proanthocyanidins and the other dimers and trimers. The standard compounds inhibited conjugated diene hydroperoxide formation by 83–89% and the lingonberry monomeric fraction by 69% at a concentration of 4.2 μg/mL and 82% at 8.4 μg/mL . The di- and trimeric fraction was a slightly more efficient antioxidant. Our results are in agreement with these previous results. Määttä-Riihinen et al. (2005) tested the antioxidant activity of the monomeric and oligomeric fraction of lingonberry and cranberry proanthocyanidins in an emulsion model system. The effects of lingonberry fractions were equal; the inhibition of lipid oxidation was 84–85%, while the oligomeric fraction of cranberries was more potent (87%) compared to the monomeric fraction (80%).

Similarly, regarding the radical scavenging activity of other proanthocyanidin sources, such as cocoa and almonds (Muselík et al. 2007; Calderón et al. 2009) and grape seeds (Spranger et al. 2008), a higher degree of polymerization resulted in a higher level of efficacy. Da Silva Porto et al. (2003) also reported that a higher degree of polymerization enhances the antioxidant activity of proanthocyanidins in LDL. This is possibly due to the increased number of OH groups (Idowu et al. 2010). On the other hand, this may be because of the interaction of proanthocyanidins with phospholipid polar head groups, which is associated with the inhibition of lipid oxidation in a chain length–dependent manner (Verstraeten et al. 2003), i.e., a higher DP of proanthocyanidins provides more protection. It has been postulated that the greater antioxidant activity of dimeric and oligomeric proanthocyanidins is due to the increasing electron delocalization of the phenyl radical by interflavan linkage (Ursini et al. 2001).

The dimeric and trimeric fraction of cranberry proanthocyanidins was the most effective of cranberry fractions, while the oligomers and polymers of the lingonberry were more effective than the dimers and trimers of lingonberry proanthocyanidins.

Cloudberries

Cloudberry phenolics, in the liposome oxidation test, showed somewhat lower lipid oxidation rates than rowanberry, cranberry, and lingonberry phenolics. The hexanal formation inhibition was 80%, whereas the other berries studied inhibited over 90% at a concentration of 8.4 μg/mL. Only a few studies have been conducted with cloudberry phenolics (Mylnikov et al. 2005; Rey et al. 2005; Kähkönen et al. 2011). Most of the investigations on ellagitannin-rich berries have been done with raspberries or blackberries. However, Kähkönen et al. (2011) tested the antioxidant activity of cloudberry in various oxidation models such as methyl linoleate, emulsion, and LDL. Cloudberry extract displayed strong antioxidant activity in methyl linoleate, while cloudberry ellagitannin isolate had only moderate activity. On the other hand, ellagitannin isolate was an excellent antioxidant in the emulsion oxidation model. Rey et al. (2005) demonstrated that cloudberry extract was as effective as quercetin in retarding the lipid oxidation of pork meat patties. Deighton et al. (2000) investigated a large variety of *Rubus* berries and concluded that berries with a high content of anthocyanins are more efficient antioxidants than berries low in anthocyanins. Mylnikov et al. (2005) showed similar behavior with red and yellow cloudberries; more anthocyanin-containing red cloudberries were twice as effective in FRAP assay than yellow cloudberries. In our studies, other berries were more effective antioxidants than cloudberries.

5.3.3 Antioxidant activity of encapsulated cloudberry phenolics (V)

The antioxidant activities of encapsulated and unencapsulated cloudberry extract were at the same level and remained quite unaltered during storage (p <0.05) (**Table 13**). The values of inhibition of conjugated diene and hexanal formation varied between 62−76 and 71−84%, respectively, meaning moderate antioxidant activities. Interestingly, the highest antioxidant activity was obtained with MC 5−8 after 64 days of storage at 66% RVP when the total amount of phenolic compounds almost halved and notably alterations in the phenolic profile was observed (**Figure 11**).

Phenolic content did not go hand in hand with antioxidant activity, since a decrease in phenolic content during storage did not seem to affect antioxidant activities. A similar phenomenon has been observed in other studies. Mullen et al. (2002) found that during storage at 4 °C for 3 days and 18 °C for 24 h, the phenolic content of raspberries varied, but no changes were observed in antioxidant activities. In another study, despite the changes in phenolic content, the antioxidant activity of strawberry purees decreased only slightly when stored at 22 °C for 16 weeks (Aaby et al. 2007). García-Alonso et al. (2003) described that phenolic content in berry dessert diminished during one year of storage at 8, 21, and 30 °C while the total antioxidant activity of dessert remained practically unaltered (at 8 °C) or decreased slightly (at 21 and 30 °C). For the anthocyanin extracts, the storage stability was improved by encapsulation into maltodextrins (Robert et al. 2010; Estupiñan et al. 2011).

Table 13. Antioxidant activity of unencapsulated and encapsulated cloudberry phenolics in a lecithin liposome oxidation model (Inhibition ± SD).

Storage		Unencapsulated		MC 5-8		MC 18.5	
RVP (%)	Time (days)	Conjugated dienes (%)	Hexanal (%)	Conjugated dienes (%)	Hexanal (%)	Conjugated dienes (%)	Hexanal (%)
0	0	67.9 ± 1.1 ^{a}	79.7 ± 0.9 ^{a}	68.8 ± 0.2 ^{a}	78.7 ± 3.5 ^{a}	68.5 ± 1.3 ^{a}	81.0 ± 1.1 ^{a}
	32	66.5 ± 4.7 ^{a}	74.4 ± 4.5 ^{a}	66.3 ± 9.5 ^{a}	77.4 ± 5.3 ^{a}	63.3 ± 7.0 ^{a}	72.6 ± 4.6 ^{b}
	64	70.0 ± 4.5 ^{a}	78.8 ± 3.6 ^{a}	71.3 ± 3.3 ^{a}	79.1 ± 2.8 ^{a}	69.6 ± 3.8 ^{a}	78.3 ± 2.6 ^{ab}
33	0	67.9 ± 1.1 ^{a}	79.7 ± 0.9 ^{a}	68.8 ± 0.2 ^{ab}	78.7 ± 3.5 ^{ab}	68.5 ± 1.3 ^{a}	81.0 ± 1.1 ^{a}
	32	63.7 ± 8.0 ^{a}	72.4 ± 5.0 ^{a}	62.3 ± 4.7 ^{b}	71.2 ± 4.8 ^{b}	62.1 ± 5.2 ^{a}	72.1 ± 3.6 ^{b}
	64	72.2 ± 4.7 ^{a}	80.3 ± 3.9 ^{a}	74.9 ± 1.4 ^{a}	81.6 ± 0.7 ^{a}	66.8 ± 5.1 ^{a}	74.8 ± 3.2 ^{ab}
66	0	67.9 ± 1.1 ^{a}	79.7 ± 0.9 ^{a}	68.8 ± 0.2 ^{ab}	78.7 ± 3.5 ^{b}	68.5 ± 1.3 ^{a}	81.0 ± 1.1 ^{a}
	32	69.2 ± 3.1 ^{a}	76.8 ± 2.0 ^{a}	68.1 ± 3.4 ^{b}	76.3 ± 2.5 ^{b}	63.6 ± 5.6 ^{a}	72.7 ± 3.1 ^{b}
	64	73.0 ± 1.6 ^{a}	79.7 ± 0.5 ^{a}	75.8 ± 4.1 ^{a}	84.3 ± 1.9 ^{a}	72.1 ± 3.1 ^{a}	80.6 ± 2.7 ^{a}

The anthocyanin profile of Andes berries was preserved best when the berries were encapsulated and stored in the absence of light. In addition, maltodextrins protected them in the presence of light compared to unencapsulated anthocyanin extract (Estupiñan et al. 2011). The antioxidant activity was best maintained with encapsulated material stored in the dark, whereas storage in light caused a slight decrease due to the decrease in the phenolic content during the storage (Estupiñan et al. 2011). In our study, encapsulation did not enhance the antioxidant activity of cloudberry phenolics. The antioxidant activity remained at the same level during the storage period. Robert et al. (2010) found similar protective behavior with pomegranate extract and juice. Pomegranates consist of anthocyanins, hydrolysable tannins, catechins, and flavonols. Maltodextrins better enhanced the stability of pomegranate phenolics than that of soybean protein isolate (Robert et al. 2010).

The minor changes in antioxidant activities may be explained by the alteration in the phenolic profiles. The major changes in phenolic profiles were observed among the proportions of ellagitannins, ellagic acids, proanthocyanidins, and hydroxycinnamic acids. All of these phenolics are known to possess antioxidant activities. For example, Zafrilla et al. (2001) reported that in raspberry jams, the antioxidant activity of ellagic acid was similar to that of gallic acid, catechins, and kaempferol but higher than that of caffeic acid and ferulic acid. Phenolic compounds are typically team players, meaning that they work synergistically by supporting each other's antioxidant activities (Nicoli et al. 1999). Phenolics formed with equal or improved antioxidant activities might compensate for the loss of original phenolics (Nicoli et al. 1999). For example, oxidation of hydrolysable tannins, e.g., ellagitannins, can lead to oligomerization through phenolic coupling, which consequently increases the number of reactive sites and enhanced antioxidant activity (Bors and Michel 2002). Moreover, the hydrolysis of ellagitannins may improve the antioxidant activity by increasing the number of free hydroxyl groups (Aaby et al. 2007).

6. CONCLUSIONS

Phenolics isolated from wild and cultivated rowanberries, cranberries, lingonberries, and cloudberries were characterized and identified prior to antioxidant activity testing in food oxidation models. The main phenolic class in rowanberry cultivars was hydroxycinnamic acids, with chlorogenic acid and neochlorogenic acid being the most abundant. In different cultivars, the contents of flavonols, proanthocyanidins, and hydroxybenzoic acids were the same. Only the contents of chlorogenic acids and anthocyanins varied from species to species; the more chlorogenic acids, the less anthocyanins. The phenolic profiles of *Vaccinium* berries cranberry and lingonberry were similar, while the profile of cloudberries differed. The proportion of anthocyanins was higher in cranberries and lingonberries than in cloudberries. The main differences were in the contents of proanthocyanidins and ellagitannins. Cranberries and lingonberries consisted of over 60% proanthocyanidins, whereas in cloudberries, the proanthocyanidin content was only 10%. Cloudberries were abundant in ellagitannins and ellagic acids, which were lacking in cranberries and lingonberries. Further compositional analysis revealed that the polymeric fraction of proanthocyanidins in lingonberries and cranberries was the most abundant, followed by the oligomeric and dimeric fractions. Lingonberry proanthocyanidins consisted only of procyanidins, while cranberries were also found to contain propelargonidins. Rare A-type dimers and trimers were detected in both cranberries and lingonberries. The content of A-type dimers was higher in lingonberries than in cranberries. On the other hand, higher amounts A-type trimers were found in cranberries. In total, the lingonberry was richer in A-type proanthocyanidins than the European cranberry. The structural analysis of cloudberry ellagitannins showed that lambertianin C and sanguiin H-10 were the main ellagitannins. Other ellagitannins were also found, such as sanguiin isomers H-2 and H-6. Among the phenolics in cloudberries, the ellagic acid content was substantial.

These berries, rich in different types of phenolic compounds, including hydroxycinnamic acids, proanthocyanidins, and ellagitannins, showed the ability to retard lipid oxidation in liposome and emulsion oxidation assays. Antioxidant activities and radical scavenging capacities were also studied with hydroxycinnamic acid glycosides. Of the hydroxycinnamic acid glycosides, sinapic acid derivatives were the most effective in preventing lipid oxidation in emulsions and liposomes and scavenging radicals in DPPH assay. In liposomes and emulsions, the hexanal formation was inhibited more than the conjugated diene hydroperoxide formation by hydroxycinnamic acid derivatives, indicating that they are particularly chain-breaking antioxidants rather than metal chelators, although they possess chelating activity as well. Berry phenolics were powerful antioxidants in liposome and emulsion models. No statistical difference was found between rowanberry cultivars, regardless of the differences in their phenolic composition. In liposomes, rowanberries were slightly more effective antioxidants than cranberry and lingonberry phenolic extracts. Greater differences were found when comparing proanthocyanidin fractions. In liposomes, the dimeric and trimeric fraction of both cranberries and lingonberries was most potent at inhibiting lipid oxidation.

Encapsulation with maltodextrins was investigated with cloudberry ellagitannins. Ellagitannins were better preserved in MC 5-8 microencapsules than in MC 18.5 microencapsules or unencapsulated. The best preservation was achieved when the capsules were stored at 0 or 33%

RVP. Even though dramatic changes could be seen after storage, different storage conditions did not affect the antioxidant activity, mainly because of the hydrolysis of ellagitannins to ellagic acids, which also have antioxidant properties.

The isolated phenolic compounds from rowanberries showed the strongest antioxidant activity in food oxidation models, which may be due to the high content of hydroxycinnamic acids, especially chlorogenic acids. In addition, cranberry and lingonberry proanthocyanidins, as well as cloudberry ellagitannins, were efficient in preventing lipid oxidation, even though slight differences in the antioxidant activities were found. The current results may be of use in improving the oxidative stability of food products by using berries as natural antioxidants.

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