DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

Non-volatile Bioactive and Sensory Compounds in Berries and Leaves of Sea Buckthorn (*Hippophaë rhamnoides*)

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In memory of my father

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ABSTRACT

Sea buckthorn (*Hippophaë rhamnoides* L., SB) is regarded as a raw material of high economic value and a source of many health-related bioactive compounds, such as flavonol glycosides (FGs), proanthocyanidins (PAs) and ethyl *β*-D-glucopyranoside (EG) in berries and ellagitannins (ETs) in leaves. These phenolic compounds are also well known components in various foods inducing astringent and bitter sensations. Ethyl *β*-D-glucopyranoside, an alkylated glucose, is among the major chemotaxonomic characteristics in SB berries.

The aims of the current work were: 1) To qualitatively and quantitatively analyze FGs, PAs in SB berries and purees, as well as FGs and ETs in SB leaf and leaf tea-type beverages; 2) To determine the effects of genetic background, growth sites and processing methods on the contents and compositions of these compounds; 3) To investigate the roles of FGs, PAs and EGs in the sensory quality of SB purees/juices; 4) To study the antioxidant activities (AAs) in leaf tea-type infusions, the correlations with AAs, FGs and ETs.

Twenty-six flavonol glycosides with isorhamnetin and quercetin as the major aglycones were found in the wild SB (ssp. *sinensis*) berries from China and cultivated berries (ssp. *mongolica*) from Finland and Canada. The contents of FGs varied from 23 to 250 mg/100 g fresh berries, which were significantly higher in ssp. *sinensis* than in ssp. *mongolica*. The berries of 'Oranzhevaya' and 'Prevoshodnaya' had the lowest (23 mg/100 g) and the highest content of FGs (80 mg/100 g), respectively. The samples from Kittilä (North Finland) had higher levels of most FGs than those from Turku (South Finland) and Québec (Canada). Among the ssp. *sinensis* berries, the berries from Sichuan had the highest contents and unique profiles of FGs. Increasing trends were detected in the contents of most FGs as the altitude increased and as the latitude decreased.

The role of ethyl *β*-D-glucopyranoside was investigated in the sensory profiles of SB juices of 'Terhi' and 'Tytti'. The taste threshold of pure EG was estimated in water solution as 1.1 ± 1.3 g/L, and the suprathreshold aqueous EG solution (5.0 g/L) was perceived mainly as bitter. Addition of EG increased bitterness of SB juice, which correlated with the EG content, as well as with the ratios of EG/acids and EG/sugars. The roles of FGs and PAs were also investigated in purees of six SB cultivars. The sensory profiles of the purees were dominated by intense sourness due to abundant malic acid, followed by astringency and bitterness. Malic acid and isorhamnetin glycosides related strongly to the astringency, whereas PA dimers, PA trimers and quercetin glycosides had less influence. Moreover, the acids/phenolic compounds ratios were more important predictors of bitterness than the individual variables alone.

Composition and contents of flavonol glycosides and ellagitannins as well as antioxidant activities were investigated in tea-type infusions processed from sea buckthorn leaves using different drying methods. These infusions had high content of phenolic substances together with associated strong antioxidant activities, were considerably acceptable for consumers. Isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside, isorhamnetin-3-*O*-rutinoside and kaempferol-3-*O*-hexoside-7-*O*-rhamnoside were the three major FGs, and stachyurin and casuarinin were the most abundant ETs in all the samples. Significant differences were found in the contents of most ETs between the infusions of 'Terhi' and 'Tytti' ($p < 0.05$). The ET contents varied significantly among the different processing methods, whereas less effect was seen on the FGs contents. Thermal processing decreased the antioxidant activities of the infusions. Additionally, significant contents of phenolic compounds were left in the leaf residues after the hot water extractions.

SUOMENKIELINEN ABSTRAKTI

Tyrni (*Hippophaë rhamnoides* L.) on taloudellisesti tärkeä elintarvikkeiden raaka-aine. Sen marjat ovat monien terveyteen vaikuttavien bioaktiivisten yhdisteiden, kuten flavonoliglykosidien (FG), proantosyanidiinien (PA) ja etyyli-β-D-glukosidin lähde, ja kasvin lehdissä on runsaasti ellagitanniineja. Nämä fenoliset yhdisteet ovat myös tunnettuja astringoivaa suutuntumaa ja karvautta aikaansaavina komponentteja monissa elintarvikkeissa. Etyyli-β-D-glukosidi, alkyloitunut glukoosi, on yksi tyrnin marjojen merkittävimmistä, tunnusomaisista kemotaksonomisista yhdisteistä.

Tämän tutkimuksen tavoitteina oli 1) määrittää laadullisesti ja määrällisesti FG:t ja PA:t marjoista ja soseista sekä FG:t ja ET:t lehdistä ja lehdistä valmistetuista teenkaltaisista juomista, 2) määrittää perinnöllisen taustan, kasvupaikan ja käsittelymenetelmien vaikutukset yhdisteiden määriin ja koostumukseen, 3) tarkastella FG:ien, PA:ien ja EG:n merkitystä soseiden ja mehujen aistittavalle laadulle ja 4) tutkia lehdistä valmistettujen teenkaltaisten juomien antioksidanttiaktiivisuutta (AA) ja yhteyksiä AA:ien ja FG:ien sekä ET:ien välillä.

26 flavonoliglykosidia, joiden pääasialliset aglykonit olivat isoramnetiini tai kversetiini, havaittiin luonnonvaraisista kiinalaisista tyrneistä (alalaji *sinensis*) ja viljellyistä suomalaisista ja kanadalaisista tyrneistä (alalaji *mongolica*). Yhdisteiden kokonaispitoisuudet vaihtelivat tuoreissa marjoissa välillä 23 ja 250 mg/100 g. Pitoisuudet olivat merkitsevästi suuremmat *sinensis*-alalajin kuin *mongolica*-alalajin marjoissa. ”Oranzhevaya”-lajikkeesta löytyi pienin (23 mg/100 g) ja ”Prevoshodnaya”-lajikkeesta suurin pitoisuus (80 mg/100 g) flavonoliglykosideja. Kittilän (Pohjois-Suomessa) näytteissä useimpien FG:ien pitoisuudet olivat korkeampia kuin Turun (Etelä-Suomessa) tai Quebecin (Kanada) näytteissä. Sichuanin (Kiina) näytteissä oli suurin FG-pitoisuus ja yksilöllisin koostumus yhdisteitä. Useimpien FG-yhdisteiden pitoisuus kasvoi altitudin kasvaessa tai leveysasteen pienentyessä.

EG:n merkitystä aistittavaan laatuun tarkasteltiin ”Terhi”- ja ”Tytti”-lajikkeista valmistetuissa mehuissa. Puhtaan EG:n makukynnykseksi vedessä määritettiin 1,1 ± 1,3 g/L ja kynnystä korkeamman pitoisuuden (5,0 g/L) vesiliuos aistittiin pääasiassa karvaana. EG:n lisäys nosti tyrnimehun karvautta, mikä korreloi EG:n pitoisuuden sekä EG:n ja happojen ja EG:n ja sokerien pitoisuuksien suhteiden kanssa. FG:ien ja PA:ien merkitystä tarkasteltiin kuudesta tyrnilajikkeesta valmistetuissa soseissa. Soseiden aistittavan laadun profiilia hallitsi voimakas omenahaposta johtuva happamuus sekä seuraavina astringoivuus ja karvaus. Omenahappo ja isoramnetiinin glykosidit olivat yhteydessä astringoivuuteen kun puolestaan PA:n dimeerit tai trimeerit sekä kversetiinin glykosidit olivat vähemmän merkittäviä. Lisäksi happojen ja fenolisten yhdisteiden suhde oli merkittävämpi karvautta ennustava tekijä kuin yksittäiset muuttujat yksinään.

Tyrnin lehdistä erilaisilla kuivausmenetelmillä valmistetuista teentyyppisistä haudukkeista tarkasteltiin flavonoliglykosidien ja ellagitanniinien koostumusta ja pitoisuuksia sekä antioksidatiivisia vaikutuksia. Kuluttajat kokivat haudukkeet hyväksyttäviksi. Niissä oli korkeita pitoisuuksia antioksidatiivisuuteen yhdistettyjä yhdisteitä. Isoramnetiini-3-*O*-glukosidi-7-*O*-ramnosidi, isoramnetiini-3-*O*-rutinosidi ja kemferoli-3-*O*-heksosidi-7-*O*-ramnosidi olivat merkittävimmät FG:t ja stakyuriini ja kasuariini olivat merkittävimmät ET:t kaikissa näytteissä. Tyrnilajikkeista valmistettujen haudukkeiden välillä oli merkitseviä eroja useimpien ET:ien pitoisuuksissa, jotka vaihtelivat merkitsevästi eri käsittelymenetelmien välillä, kun taas FG:ien pitoisuuksiin menetelmillä oli vähemmän vaikutusta. Lämpökäsittely vähensi haudukkeiden antioksidatiivista aktiivisuutta. Lisäksi merkittäviä pitoisuuksia fenolisia yhdisteitä jäi lehtien jäännöksiin kuumavesiuuton jälkeen.

LIST OF ABBREVIATIONS

2-DR	2-deoxyribose
AAs	Antioxidant activities
ANOVA	Analysis of variance
BET	Best estimate of individual threshold
BL-DMAC	Brunswick Laboratories 4-dimethylaminocinnamaldehyde
DP	Degree of polymerization
DW	Dry weight
EA	Ellagic acid
EDTA	Ethylenediaminetetraacetic acid
EG	Ethyl β-D-glucopyranoside
ESI-MS	Electron spray ion-mass spectrometer
ETs	Ellagitannins
FA	Flavonol aglycone
FG	Flavonol glycoside
FW	Fresh weight
G	Glucose
GC-TMS	Gas chromatography as trimethylsilyl
He	Hexoside
HHDP	Hexahydroxydiphenoyl
HPLC	High-performance liquid chromatography
Is	Isorhamnetin
ka	kaempferol
L-PLS	L-shaped partial least regression
PAs	Proanthocyanidins
PCs	Procyanidins
PCA	Principal component analysis
PDs	Prodelphinidins
Pe	Pentoside
PKD2L1	Polycystic kidney disease 2-like 1
PLS	Partial least regression
PME	Pectinmethylesterase
R	Rutinoside
Rh	Rhamnoside
S	Sophoroside
SB	Sea buckthorn
SC-CO_2	Supercritical carbon dioxide
SIR	Selected ion recording

LIST OF ORIGINAL PUBLICATIONS

I. Ma, X.; Laaksonen, O.; Zheng, J.; Yang, W.; Trépanier, M.; Kallio, H.; Yang, B. (2016) Flavonol glycosides in berries of two major subspecies of sea buckthorn (*Hippophaë rhamnoides* L.) and influence of growth sites. *Food Chemistry*, *200*, 189–198.

II. Ma, X.; Laaksonen, O.; Heinonen, J.; Sainio, T.; Kallio, H.; Yang, B. (2017) Sensory profile of ethyl *β*-D-glucopyranoside and its contribution to quality of sea buckthorn (*Hippophaë rhamnoides* L.). *Food Chemistry*, *233*, 263−272.

III. Ma, X.; Yang, W.; Laaksonen, O.; Nylander, M.; Kallio, H.; Yang, B. (2017) Role of flavonols and proanthocyanidins in the sensory quality of sea buckthorn (*Hippophaë rhamnoides* L.) berries. *Journal of Agricultural and Food Chemistry*, *65*, 9871−9879.

IV. Ma, X.; Moilanen, J.; Laaksonen, O.; Yang, W.; Tenhu, E.; Yang, B. (2019) Phenolic compounds and antioxidant activities of tea-type infusions processed from sea buckthorn (*Hippophaë rhamnoides*) leaves. *Food Chemistry*, *272*, 1−11

1 INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.) widely distributed in Asia and Europe, is a highly valued plant, due to its nutritional, phyto-therapeutic and environmental values (Bal, Meda, Naik & Satya, 2011, Li & Schroeder, 1996). Eight subspecies are included in the *H. rhamnoides* (Sun et al., 2002), among which ssp. *sinensis* and ssp. *mongolica* are the most abundant and commercially interesting. The female cultivars 'Terhi' and 'Tytti', originating from Finnish wild strains of ssp. *rhamnoides,* were bred and cultivated in 2000. Due to their characteristics of winter-hardiness, resistance to disease (e.g. stem canker) and moderate growth, 'Terhi' and 'Tytti' have become the main SB cultivars in Finland (Karhu, 2003). The berries and leaves of SB are both rich sources of bioactive components, such as flavonol glycosides (FGs), proanthocyanidins (PAs), ellagitannins (ETs), phenolic acids, phytosterols and fatty acids (Bal, Meda, Naik & Satya, 2011, Hellström, Pihlava, Marnila, Mattila & Kauppinen, 2013, Pop et al., 2014, Teleszko, Wojdyło, Rudzińska, Oszmiański & Golis, 2015). These compounds may promote human health mainly through anti-oxidative (Gao, Ohlander, Jeppsson, Björk & Trajkovski, 2000), anti-inflammatory (Yang & Kortesniemi, 2015), radioprotective (Chawla et al., 2007), tissue repairing (Geetha, Jayamurthy, Pal, Pandey, Kumar & Sawhney, 2008), and cardiovascular effects (Suomela, Ahotupa, Yang, Vasankari & Kallio, 2006).

Flavonols, mainly as glycosides, constitute the main group of flavonoids present in SB berries (Teleszko et al., 2015), which also contain notable amounts of PAs (Hellström, Törrönen & Mattila, 2009, Yang, Laaksonen, Kallio & Yang, 2016a). The content of the phenolic compounds in the berry varies according to origin, weather condition, growth location, latitude and harvest date (Kortesniemi, Sinkkonen, Yang & Kallio, 2017, Kortesniemi, Sinkkonen, Yang & Kallio, 2014, Ma et al., 2016, Yang et al., 2009, Yang et al., 2016a, Yang, Laaksonen, Kallio & Yang, 2016b, Zheng, Kallio & Yang, 2016). FGs have been found to induce an astringent sensation even at very low concentrations in sensory evaluations (Hufnagel & Hofmann, 2008a, Scharbert, Holzmann & Hofmann, 2004). However, flavonol aglycones (FAs) may be perceived as bitter due to activation of taste receptors of bitterness (Roland et al., 2013). PAs have an important role in the astringency and bitterness of plant-based foods and beverages. Although PAs are found less abundant than FGs in SB berries, they may play a significant role in the astringent and bitter sensations due to their relatively low threshold values (Hufnagel & Hofmann, 2008a, Laaksonen et al., 2015, Schwarz & Hofmann, 2007).

SB berry pulp was found to contain a high content of phenolic acids (Arimboor, Kumar & Arumughan, 2008). Salicylic acid was the dominant one in SB berries, accounting up to 74 % of the total phenolic acids in six Europe cultivars (Zadernowski, Naczk, Czaplicki, Rubinskiene & Szałkiewicz, 2005a).

Sea buckthorn berries contain ethyl *β*-D-glucopyranoside (EG), and its content along with other sugars varies significantly with genotype, harvesting time, and origin (Tiitinen et al., 2006, Ohkawa et al, 2009, Yang, 2009, Zheng et al., 2011 and Zheng et al., 2012). In some berries of ssp. *rhamnoides*, the content of EG is up to 1.9 g/100 ml juice, dominating the sugar fraction (Yang, 2009). Moreover, EG has been found in human plasma and urine after a SB meal based on an NMR metabolomics study (Lindstedt et al., 2014). Thus, the content of EG in berries is among the major chemotaxonomic characteristics in SB.

Additionally, sea buckthorn berries also contain oil in the seeds, the fruit pulp and peel. SB have primarily linoleic (18:2n−6) and α-linolenic (18:3n−3) acids with n−6/n−3 ratios close to 1 (Yang et al., 2011). It has been reported that SB oil can relieve symptoms of dry eyes (Jarvinen et al., 2011, Larmo et al., 2010).

Despite the widely shown health-beneficial effects, berries are not commonly consumed in the US and Europe. Orosensory properties of food are the most important drivers of consumer preferences, choices and acceptance (Drewnowski, 1997, Geertsen, Allesen-Holm, Byrne & Giacalone, 2016). Therefore, improving the sensory properties is essential to increase intake of certain food such as berries and berry products. SB berries have a unique taste, which is typically described as sour, astringent and bitter, with a very low degree of sweet characteristics. Generally, the contents of sugars and acids, as well as the ratio between sugar and acid play a crucial role in determining the flavor and consumer acceptance of berries and berry products (Laaksonen et al., 2013, Tiitinen, Hakala & Kallio, 2005). The utilization of berries is often limited as food and food ingredients due to negative features, such as bitter or astringent properties (Laaksonen, Knaapila, Niva, Deegan & Sandell, 2016). Sensory properties of SB berries are among the important criteria for cultivation, breeding and industrial utilization of sea buckthorn.

Sea buckthorn leaves are alternate, narrow-lanceolate with a silver-grey color on the bottom side. In ancient Greece, the SB leaves have been used to feed horse for weight gain and shiny hair. Recently, SB leaf extracts have been scientifically investigated and various pharmacological activities have been reported including anti-inflammatory, immunomodulatory, radio protective, adaptogenic activity (non-specific resistance of the body) and tissue regenerative properties (Ganju et al., 2005, Geetha, Singh, Ram, Ilavazhagan, Banerjee & Sawhney, 2005, Gupta, Upadhyay, Sawhney & Kumar, 2008, Gupta et al., 2005, Saggu et al., 2007). Moreover, the extracts of SB leaves have been used in the treatment of colitis, diarrhea and enterocolitis in humans and animals (Guliyev, Gul & Yildirim, 2004, Tsybikova et al., 1983). Only small amounts of SB leaves are applied as raw materials to produce fodder, beverages and nutraceuticals in some European countries, and the majority of SB leaves has remained as agricultural wastes after berry harvesting.

SB leaves have several natural advantages, such as large production volume, easy to collect, length of acquisition cycle, simple procedures for production and processing, and easy storage. Hence, increased exploitation of SB leaves will help farmers to improve profitability and to promote the sustainable agriculture and the rational use of SB resources. SB leaves are rich in isorhamnetin and quercetin glycosides (Pop et al., 2013). FGs are of interest because of their evident health benefits, such as antioxidative capacity against free radicals, lower incidence of type 2 diabetes, and reducing the risk of thrombogenesis and coronary heart disease (Chen & Chen, 2013, Cheng et al., 2003, Jacques, Cassidy, Rogers, Peterson, Meigs & Dwyer, 2013). Other groups of well-known plant phenolics found in SB leaves are hydrolyzable tannins and more specifically ETs. The content of ETs is high in SB leaves, more than 100 mg/g DW (Suvanto et al., 2018, Tian et al., 2017). Like other polyphenols, ETs also possess a wide range of biological activities, such as antioxidative functions, anti-inflammatory activities, and prebiotic effects (Landete, 2011). In order to provide a better understanding of potential health benefits, identification and quantitative determination of phenolic compounds are of special importance in the SB leaves and related products.

In the first part of this doctoral thesis, a literature review is presented, summarizing the recent publications on chemical characteristics, health effects, sensory properties and biological activities of phenolic and non-phenolic compounds in SB berries and leaves. Furthermore, the review also covers the challenges of development and processing of SB products.

In the experimental part of the doctoral thesis, SB berries of two subspecies (ssp. *mongolica* and ssp. *sinensis*) were compared based on the content and composition of FGs in eleven inconsecutive years. The effects of genetic background, growth sites, altitude and latitude on flavonol composition and content were studied. Also the taste threshold of EG in water was determined, and the roles of EG, FGs and PAs in the sensory quality of SB juices/purees were investigated, respectively, especially in the astringent properties and bitterness. In the study of ssp. *rhamnoides* leaves, the compositions and contents of FGs and ETs, and AAs were investigated in tea-type infusions. And the influence of cultivars (‘Terhi’ and ‘Tytti’) and processing methods on contents and compositions of ETs and FGs were studied. Possible association between AAs and FGs as well as ETs was explored. In addition, “tea” residues and fresh leaves were also studied.

2 REVIEW OF THE LITERATURE

2.1 Compounds of sea buckthorn associated with potential health effects

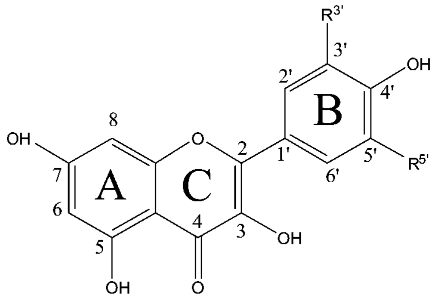
Improvement in knowledge on the role of foods in human health has increased consumer attention on various nutraceuticals and health promoting foods. Berries and berry products may be an important part of a healthy diet because of the high content of bioactive compounds. Sea buckthorn is a rich source of a wide variety of non-nutritive and nutritive components, such as sugars, carotenoids, vitamins, minerals, flavonoids, phenolic acids, and tannins. The following is a comprehensive review of the main bioactive compounds in sea buckthorn, including their structures, absorption, and biological activities and potential effect on human health.

2.1.1 Flavonol glycosides

Flavonol glycosides are a group of flavonoids, which are widely distributed in fruits and vegetables. It has been reported that FGs may have a potential role in reducing the risk and managing of chronic diseases such as cancers, diabetes and cardiovascular diseases (Andrae-Marobela, Ghislain, Okatch & Majinda, 2013, Bal, Meda, Naik & Satya, 2011, Hertog et al., 1995, Jacques, Cassidy, Rogers, Peterson, Meigs & Dwyer, 2013). Some studies have shown that increasing dietary intake of FGs of SB berries may reduce cardio-vascular mortality (Cheng, Kondo, Suzuki, Ikeda, Meng & Umemura, 2003, Clair, Yang, Raija, Heikki, Gerald & Minihane, 2002).

The structure of a flavonol consists of two aromatic rings and one heterocyclic pyran ring (C6–C3–C6) (**Figure 1**) and has a double bond between C2 and C3, and a keto group at C4 as well as a hydroxyl group at C3. The antioxidant activity of flavonoids depends on the location and number of free hydroxyl groups in their skeleton. For instance, in flavonols, the number and pattern of hydroxyl groups in the A and B rings are major factors determining the free radical scavenging potential of the compounds (Nabavi, Nabavi, Eslami & Moghaddam, 2012).

Flavonols such as kaempferol, quercetin, myricetin and isorhamnetin exist in plant foods as sugar conjugates (**Figure 1**). The aglycones can be glycosylated with even up to four or more saccharides. The most common monosaccharides present in the sugar moieties of flavonol glycosides are glucose (G), galactose, rhamnose (Rh) and xylose, whereas typical disaccharides are sophorose (S) and rutinose (R) (Veitch & Grayer, 2008). The preferred site of glycosylation is C3, and less frequently the position C7. The presence of various saccharides and complex linkages (e.g. acylated compounds) results in an enormous number of diverse FGs in plant foods.



Name	$R^{3'}$	$R^{5'}$
Isorhamnetin	OCH_3	H
Kaempferol	H	H
Quercetin	OH	H
Myricetin	OH	OH

Figure 1. Structures of flavonol aglycones.

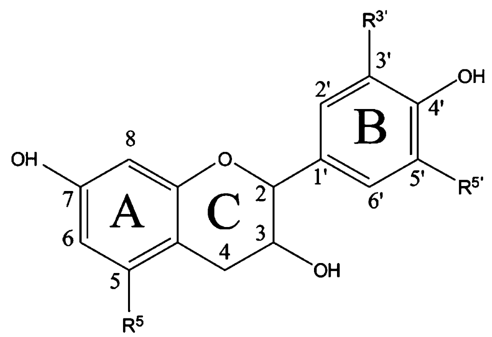
Flavonol glycosides form the biggest class of phenolic compounds in SB berries (Teleszko, Wojdyło, Rudzińska, Oszmiański & Golis, 2015). Isorhamnetin is the typical and most abundant aglycone in FGs in sea buckthorn, and isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside are the two major FGs in the berries studied (Ma et al., 2016, Zheng, Kallio & Yang, 2016). Low quantities of quercetin and kaempferol glycosides are also found in the berries (Pop et al., 2013, Rösch, Krumbein, Mügge & Kroh, 2004). Isorhamnetin is less common in fruits and berries than kaempferol and quercetin (Belitz, Grosch & Schieberle, 2004). However, it has been reported that isorhamnetin-3-*O*-rutinoside is the major phenolic compound in copao fruits (*Eulychnia acida* Phil.) in the family Cactaceae (Jiménez-Aspee et al., 2014). Cocoa, onions and berries contain quercetin glycosides in abundance, which are also found in olive oil, red wine, and tea (Tan et al., 2003). A high concentration of kaempferol is found in leafy vegetables, such as spinach, kale, endive and fennel. Leaves of SB also contain abundant flavonols. Pop et al. reported that SB leaves are rich in FGs with content up to 11.2 mg/g DW. The glycosides of isorhamnetin, quercetin and kaempferol are the major FGs in the extract of SB leaves (Pop et al., 2013, Tian et al., 2017, Zu, Li, Fu & Zhao, 2006).

Flavonols are bioactive compounds associated with human health. Quercetin is the main flavonol in the human diet (Ahn, Lee, Kim, Park & Ha, 2008). In a Finnish cohort, quercetin constituted on average about 95% of the flavonoid intake (Knekt, Jarvinen, Reunanen & Maatela, 1996). Quercetin is known as a potent natural antioxidant which inhibited oxidative stress under *in vitro* and *in vivo* conditions (Nabavi, Nabavi, Eslami & Moghaddam, 2012). Free radicals and lipid peroxides can be quenched directly by quercetin. Moreover, quercetin indirectly enhances the generation of non-enzymatic antioxidants and also increases the activities of antioxidant enzymes *in vivo* (Nabavi, Nabavi, Eslami & Moghaddam, 2012). Recently, circumstantial evidence suggests that quercetin is able to ameliorate obesity through some molecular pathways (Nabavi, Russo, Daglia & Nabavi, 2015). Yang et al. also found the potential of isorhamnetin in protecting hepatocytes against oxidative stress by Nrf2 activation and in inducing the expressions of its downstream genes (Yang et al., 2014). It has been further shown that kaempferol glycosides have an anti-obesity and anti-diabetic potential through reducing the accumulation of adipose tissue resulting in improvement of hyperlipidemia as well as diabetes in obese mice (Zang, Zhang, Igarashi & Yu, 2015). Despite the wide range of pharmacological activities of quercetin, it is important to note that there are some limitations for clinical trials (oral administration) on quercetin. Due to its narrow therapeutic dose-range *in vitro,* the risk of neurotoxicity is not negligible (Ossola, Kääriäinen & Männistö, 2009).

In foods, glycosides are the main forms of flavonol compounds. The chemical structure of the flavonol aglycones and the total number of sugar moieties, have an effect on the bioavailability and bioactivity related to the digestion stage (Manach, Scalbert, Morand, Remesy & Jimenez, 2004). In the antioxidant assay of tea infusions, the quercetin mono-glycosides were more effective than the diglycosides and the triglycosides; kaempferol aglycone was about 3–10 times more effective than kaempferol glycosides (Plumb, Price & Williamson, 1999). Antunes-Ricardo et al. showed glycosylation to have a significant effect on the anti-proliferative effect of isorhamnetin (Antunes-Ricardo, Moreno-García, Gutiérrez-Uribe, Aráiz-Hernández, Alvarez & Serna-Saldivar, 2014). Diglycosides of isorhamnetin were shown to be more cytotoxic than the free aglycone or triglycosides when HT-29 cells were tested (Antunes-Ricardo, Moreno-García, Gutiérrez-Uribe, Aráiz-Hernández, Alvarez & Serna-Saldivar, 2014). Aglycones and only some glucosides can be absorbed in the small intestine. Those flavonols linked e.g. to a rhamnose moiety must reach the colon and be hydrolyzed by rhamnosidases of the microflora before absorption (Hollman & Katan, 1997). In the case of quercetin glycosides, the bioavailability of quercetin 4′-glucoside is about 5 times higher than that of 4′-rutinoside (rutin) in humans. The maximum absorption occurs 0.5–0.7 h after ingestion of quercetin 4′-glucoside and 6–9 h after ingestion of the same quantity of rutin. (Graefe et al., 2001, Hollman, Bijsman, van Gameren, Cnossen, de Vries & Katan, 1999). Similarly, due to the dominance of glucosides, absorption of quercetin after ingestion of onions is more rapid and efficient than after ingestion of apples containing a variety of glycosides and tea rich in quercetin-3-rutinoside (Hollman, van Trijp, Buysman, Mengelers, de Vries & Katan, 1997).

2.1.2 Proanthocyanidins

Tannins comprise both condensed tannins, known as proanthocyanidins (PAs), and hydrolysable tannins. PAs are oligomers and polymers consisting of flavan-3-ol units produced *via* the biosynthetic pathway of flavonoids. Several reviews provide excellent summaries of the agricultural benefits (Aerts, Barry & McNabb, 1999), chemistry (Ferreira & Slade, 2002), and biochemistry (Marles, Ray & Gruber, 2003) of PAs. In recent years, there has been an increasing interest in PAs due to their potentially beneficial effects on human health, and the important sensory properties of fresh fruit and derived products (e.g. wine).



Proanthocyanidins (PAs)	Flavan-3-ol	$R^{3'}$	$R^{5'}$	R^{5}
Procyanidins (PCs)	(epi)catechin	OH	H	OH
Prodelphinidins (PDs)	(epi)gallocatechin	OH	OH	OH
Proguibourtinidins (PGs)	(epi)guibourtinidol	H	H	H

Figure 2. Structures of flavan-3-ol units.

The flavan-3-ol units have the typical C6–C3–C6 flavonoid skeleton. The structure of flavan-3-ols is shown in **Figure 2**, in which the two benzene rings and a heterocyclic dihydropyran ring are distinguished by the letters A, B and C. PAs differ structurally according to the hydroxylation pattern and stereochemistry of the flavan-3-ol units, as well as the type of linkages between monomeric units and the numbers of the monomeric units, also known as the degree of polymerization in the molecule. The flavan-3-ol units with a 3′, 5′, -dihydroxy substitution and a 3′, 5′, 5-trihydroxy substitution are called (epi)catechin and (epi)gallocatechin, respectively. PAs consisting of only (epi)catechin or (epi)gallocatechin are known as procyanidins (PCs) and prodelphinidins (PDs), respectively. PCs, PDs or mixed PC/PDs are most common in food, such as raspberries, strawberries, almonds and some beans. The oligomers of the SB pomace consisted mainly of PD subunits whereas PCs were present in smaller amounts (Rösch, Mügge, Fogliano & Kroh, 2004). The monomeric units of flavan-3-ols are linked by C–C (*via* C4–C8 and/or C4–C6) as in the case of B-type PAs. Occasionally, additional C–O–C (C2–O–C7) bonds are present together with C-C linkage between the monomer units, thereby forming A-type proanthocyanidins. Most of the common PAs are of B-type, whereas A-type PAs are present in a few specific foods only (Deng, Xu, Zhang, Li, Gan & Li, 2013).

Recently, a rapid and sensitive method for profiling of proanthocyanidins of SB berries was established by researchers of our lab. PAs with degree of polymerization (DP) from 2 to 11 were detected (Kallio, Yang, Liu & Yang, 2014). Only B-type PAs were found with this method. The contents of dimeric, trimeric, and tetrameric were in the range of 1.4−8.9, 1.3−9.5, 1.0−7.1 mg/100 g DW, and the content of total PAs varied from 390 to 1940 mg/100 g DW among the berries of ssp. *rhamnoides,* ssp. *sinensis* and ssp. *mongolica* (Yang et al., 2016a). In ssp. *rhamnoides*, berries grown in north Finland contained higher total PAs (2−3 times) than that found in south Finland. However, the cultivars of 'Terhi' and 'Tytti' from north Finland contained lower content of oligomeric PAs than those in south Finland (Yang et al., 2016b). In north Finland, the weather conditions, including the length and radiation sum of the growth season as well as the temperature sum, were negatively correlated with the total PAs but positively with PA oligomers (Yang et al., 2016b). Arimboor et al. reported that SB leaves contained lower content PAs compared with pulp and seeds of SB berries (Arimboor & Arumughan, 2011). Due to the polymeric nature of PAs, their analysis and estimation in food is a challenging task.

Many experimental studies have suggested potential health-beneficial effects of various crude and purified PA fractions from plant tissues (**Table 1**). The already documented health effects of grapes (wines, juices and grape seed extracts) (Bagchi et al., 2000, Katiyar, Pal & Prasad, 2017) and cranberry juice (Foo, Lu, Howell & Vorsa, 2000) are further validated. It has even been proposed that proanthocyanidins of SB pomace possessed antioxidant capacities that were higher or comparable to that of ascorbic acid or Trolox (Rösch, Krumbein & Kroh, 2004). The DP did not affect significantly the antioxidant capacities of monomeric flavan-3-ols and dimeric PAs in sea buckthorn (Rösch, Krumbein & Kroh, 2004). Several clinical trials with human subjects have suggested that consumption of grape seed PAs may promote cardiovascular health *via* significant reduction of oxidized low-density lipoproteins (Vinson et al., 2002), and indirect reduction of plasma lipid hydroperoxide levels during the postprandial phase (Natella, Belelli, Gentili, Ursini & Scaccini, 2002).

The bioavailability, antinutrition and potential toxicity of PAs are also an important issue. The DP value affects largely the absorption of PAs. After a PA-rich diet, PA dimers have been detected in human plasma but their absorption is less than that of the flavanol monomer. PAs are poorly absorbed in the gut, and polymers with DP > 4 are not absorbed at all in the small intestine (Manach, Williamson, Morand, Scalbert & Remesy, 2005). Even though the degree of polymerization greatly affects intestinal absorption of PAs, the health effects of PAs may not require efficient absorption, and the compounds that are not absorbed in the intestine still may have a positive influence on health due to their bioactivities in the gut (Teixeira, 2002). PDs may strongly bind proteins in the rumen so that the desired dissociation of the tannin–protein complex in the small intestine is inhibited (Mueller-Harvey, 2006). Thus, high relative proportions of PD may result in an antinutritional effect. Grape seed extracts rich in PAs did not show genotoxic activity through toxicity testing in animal or in cell models (Yamakoshi, Saito, Kataoka & Kikuchi, 2002). Further, as supplementation to feed, long-term consumption of PAs of grape seeds by laboratory animals did not result in apparent signs of toxicity (Vaid, Sharma & Katiyar, 2010).

Table 1. Some potential health-protective effects of proanthocyanidins (PAs).

Effect	Source	Reference
Anti-non-melanoma skin cancer	Grape seeds	Katiyar, Pal & Prasad, 2017
Inhibition of bacterial adhesion to urinary tract	Cranberry	Foo, Lu, Howell & Vorsa, 2000
Immunomodulatory	*Ecdysanthera utilis*	Lin, Kuo & Chou, 2002
Antimutagenic	*Hamamelis virginiana* bark	Dauer et al, 2003
Reduction of skin irritation	*Hamamelis virginiana* bark	Deters et al, 2001

2.1.3 Ellagitannins

Ellagitannins (ETs), which belong to the hydrolyzable tannins, are water-soluble phenolic compounds of high molecular weight. The molecular weights of ETs can be up to 4000 Da, and they precipitate proteins and alkaloids (Santos-Buelga & Scalbert, 2000). ETs constitute a complex class of polyphenols characterized by one or more hexahydroxydiphenoyl (HHDP) group, which is a basic structure of the majority of the ET monomers. When exposed to acids or bases, hydrolysis of ETs yields hexahydroxydiphenic acid, which spontaneously rearranges into the water-insoluble ellagic acid (EA). This reaction has been utilized for detection and quantification of ETs as EA equivalents after acid hydrolysis of food samples (Bate-Smith, 1962).

ETs occur naturally in certain fruits, herbs and seeds. ETs have been reported by simple class detection in extracts from various plant species of economic importance (Haslam, 1989). However, screening was largely carried out in leaves and not in the edible parts of the plants. Finnish sea buckthorn (*Hippophaë rhamnoides* ssp. *rhamnoides*) leaves are shown to have a high content of ETs, more than 100 mg/g DW (Moilanen, Koskinen & Salminen, 2015, Suvanto et al., 2018). Ten major ETs were identified and quantified in SB leaves, of these compounds, hippophaenin C, stachyurin, and casuarinin (**Figure 3**) were on average the most abundant compounds (Suvanto et al., 2018). Ellagitannins purified from SB leaves, such as strictinin, isostrictinin, and casuarictin, had been used to develop therapeutic agent. These compounds had antiviral activity against a wide spectrum of viruses, suppressed the growth of Gram-positive and Gram-negative microorganisms, and had interferon-inducing activity (Korekar et al., 2011). Moreover, hippophaenin B showed higher antioxidant activity than other ETs in SB leaves (Moilanen & Salminen, 2008). In Western diets, berries such as strawberries and raspberries are the major sources of intake of ETs. EAs are also identified in walnut and pecan nut (Daniel et al., 1989).

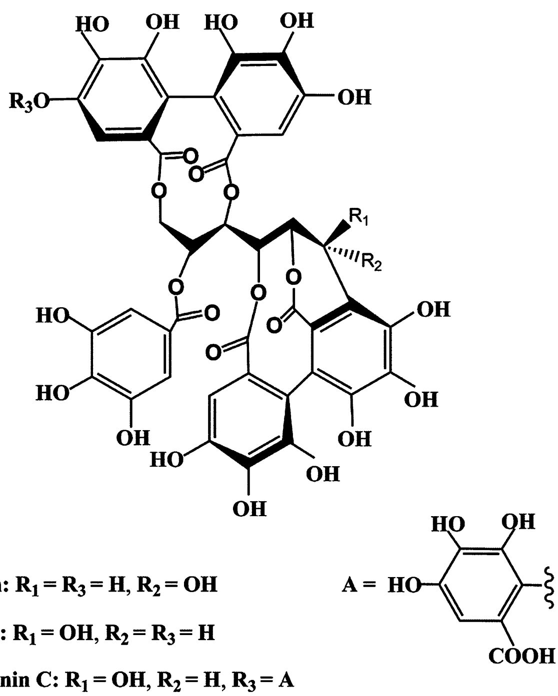


Figure 3. Examples of ellagitannins in sea buckthorn leaves.

So far, only limited information is available on the absorption and metabolism of ETs in humans. ETs undergo hydrolysis by stomach acid and intestinal enzymes to yield ellagic acid (EA), part of which is absorbed in the small intestine (Mertens-Talcott, Jilma-Stohlawetz, Rios, Hingorani & Derendorf, 2006). The unabsorbed ETs and remaining EA are further metabolized to urolithins by the microbiota in the large intestine. Metabolites of ETs in the circulatory system are among the key compounds for elucidating the protective effects of natural bioactive compounds on human health (Ludwig et al., 2015). EA is proposed to be considered as a future biomarker for human bioavailability studies related to consumption of ETs from food sources (Seeram, Lee & Heber, 2004). Current knowledge with animal studies (rats, mice and Iberian pigs) contributes more to shedding light on ET metabolism. These authors found that 10% of the EA dose given to rats was absorbed, metabolized and excreted as 3,8-dihydroxy-6H-dibenzo [*b, d*] pyran-6-one in urine (Doyle & Griffiths, 1980). Further studies showed that mice given higher doses had higher absorption rates (28%) of ETs (Teel & Martin, 1988). The ellagitannin fraction extracted from sea buckthorn revealed concentration-dependent cytotoxicity. In an *in vitro* cell culture study, ellagitannin fractions at concentrations of less than 0.2 mg/mL caused an increase in the proliferation of lymphocytes and stimulated the cytokine production, whereas in higher concentrations showed a cytotoxic effect on lymphocyte (Chernov et al., 2003).

ETs have shown both anti-herbivoric activities in plants and health-promoting activities in humans, the latter including antioxidant, antimicrobial, anticancer, and anti-inflammatory activities (Heinonen, 2007, Landete, 2011). Due to the high content of punicalagin isomers, pomegranate juice has strong antioxidant properties (Gil, Tomás-Barberán, Hess-Pierce, Holcroft & Kader, 2000). Sanguiin H-6 is also reported to be a major contributor to the antioxidant capacity together with vitamin C and anthocyanins in raspberries (Mullen et al., 2002). Furthermore, ETs and EA may play important roles in protection against certain chronic diseases according to an epidemiological study (Arts & Hollman, 2005). However, ellagitannins can inhibit protease activities at levels which could affect protein digestion in the gastrointestinal tract (Mcdougall & Stewart, 2005). Sometimes, the results *in vitro* do not match with the findings of *in vivo* studies. This may be explained by the low bioavailability of the antioxidative ETs and EA. Moreover, polyphenols are metabolized to urolithins by microbes in the gut, which have been reported as less potent antioxidants compared to the ETs (Cerdá et al., 2004). By contrast, urolithins may display health benefits, such as estrogenic and/or anti-estrogenic activity.

2.1.4 Phenolic acids

As a major class of phenolic compounds, phenolic acids are widely distributed in the human diet, particularly in fruits, vegetables, herbs, spices and beverages (coffee, beer, wine, and fruit juices)(Crozier, Jaganath & Clifford, 2009, Herrmann & Nagel, 1989). Much attention has been focused on phenolic acids, due to their relatively high concentrations in food and beverages, and strong antioxidant activity, as well as due to a relatively high rate of intestinal absorption. Dietary phenolic acids can be rapidly and extensively metabolized in humans (Nardini et al., 2006, Nardini et al., 2009).

The most commonly occurring phenolic acid is caffeic acid, which is also found in esters, such as chlorogenic acid which is esterified with an –OH group of quinic acid. Chlorogenic acid is present in many fruits, vegetables and in coffee. Other phenolic acid derivatives are hydrolyzable tannins. The phenolic acids are either gallic acid in gallotannins (mango fruit) or other phenolic acids derived from the oxidation of galloyl residues in ellagitannins (Scalbert & Williamson, 2000).

Free phenolic acids constituted only 1.3–2.3%, and phenolic acid esters up to 21.2% of the sum of phenolic acids and their derivatives in SB berries (Zadernowski, Naczk, Czaplicki, Rubinskiene & Szałkiewicz, 2005a). In a study by Zadernowski et al. (2005b), the total content of phenolic acids ranged from 3.6 to 4.4 mg/g of SB berries (DW). Phenolic acids in SB berries were found to be concentrated in seeds (approximately 70% of total phenolic acids in the whole berries), and the total phenolic acid content in seed kernel (5.7 mg/g) was higher than that in the seed coat (Arimboor, Kumar & Arumughan, 2008). Phenolic acids included gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, salicylic acid, *p*-coumaric acid, cinnamic acid, caffeic acid and ferulic acid in sea buckthorn berries and leaves, among which gallic acid was the dominant phenolic acid both in free and bound forms (Arimboor, Kumar & Arumughan, 2008).

The influences of phenolic acids on both the expression and activity of enzymes involved in the production of inflammatory mediators have been studied by using cell and animal models (Russell & Duthie, 2011, Russell et al., 2008). The cyclo-oxygenase 2, one enzyme for phenolic acids in maintaining gut health, which is strongly and rapidly induced in response to mediators of inflammation, growth factors, cytokines and endotoxins (Russell & Duthie, 2011). Moreover, phenolic acids exert a direct anti-proliferative action on T47D human breast cancer cells, even at low concentrations (Kampa et al., 2004). The information related to systemic bioavailability of phenolic acids *in vivo* is still limited. It is difficult to evaluate distinctly the correlation of dietary intake with physiological effects due to the complexity of absorption and microbial metabolism.

2.1.5 Non-phenolic compounds

2.1.5.1 Lipophilic compounds

The pulp oil of sea buckthorn has a high content of palmitoleic acid. Since this fatty acid is a major constituent of skin fat, the pulp oil is used for cosmetic products and for healing purposes. The seed oil contains high amounts of linoleic acid and α-linolenic acid (close to 1:1), which is different from the polyunsaturated fatty acid composition of major vegetable oils (Ursin, 2003, Yang & Kallio, 2001). The content of carotenoids varied from 6 to 24 mg/100 g FW in SB berries (Teleszko et al., 2015) and the range of tocopherols and tocotrienols was 8−32 mg/100 g in seeds and 6−14 mg/100 g in whole berries (Kallio et al., 2002a). Further, the seeds of ssp. *mongolica* contained more tocopherols and tocotrienols than those of ssp. *sinensis* (Kallio, Yang, Peippo, Tahvonen & Pan, 2002a). An increase in total carotenoid concentration was observed during ripening of SB berries in three German cultivars ‘Askola’, ‘Hergo’ and ‘Leikora’ (Raffo, Paoletti & Antonelli, 2004). Moreover, SB leaf also was reported to contain high contents of tocopherols (vitamin E) and carotenoids (including β-carotene, a provitamin A) (Hellström, Pihlava, Marnila, Mattila & Kauppinen, 2013).

In addition, SB oil from seeds and pulp contain large amounts of phytosterols, which included both unsaturated sterols and saturated sterols (Alasalvar & Bolling, 2015). The total content of phytosterol varied from 1.2 to 1.8 mg/g in the seeds, and from 12 to 23 mg/g in the oil of SB berries from China and Finland. *Beta*-sitosterol was the major sterol found in the seeds, constituting 57–76% of the total phytosterols in seeds (Li, Beveridge & Drover, 2007a, Sajfrtová et al., 2010, Yang et al., 2001).

In Russia and China, SB oil has been approved for clinical use in hospitals, due to numerous shown health benefits, including anti-atherogenic, cardio-protective, anti-platelet, antiulcer activities, and anti-depressive properties, which have been demonstrated using cell culture, animal models, and clinical trials in humans (Basu et al., 2007, Johansson et al., 2000, Olas, 2018, Xing et al., 2002). Phytosterols may lower serum cholesterol concentrations and have prophylactic properties against hypercholesterolemia-induced cardiovascular disorders (Olas, 2018).

2.1.5.2 Sugars, sugar alcohols and non-phenolic organic acids

Sugars and non-phenolic organic acids are the main soluble constituents of SB berries and have a crucial role in determining the sensory properties, eventually, consumer acceptability of products based on SB berries. Glucose (*α*- and *β*-D-pyranose) and fructose (*α*- and *β*-D-furanose and *β*-D-pyranose) were two major sugars in all SB berries investigated, and trace amounts of sucrose also were detected (Yang, 2009, Zheng, Yang, Trepanier & Kallio, 2012). Finnish berries of ssp. *rhamnoides* contained relatively lower content of sugars compared to the berries of other subspecies from Russia (ssp. *mongolica*) and China (ssp. *sinensis*) (Tang, Kälviäinen & Tuorila, 2001, Yang, 2009). Berries of both 'Terhi' and 'Tytti', two Finnish sea buckthorn cultivars, had a lower content of fructose compared to the wild Finnish berries of ssp. *rhamnoides* (Kortesniemi, Sinkkonen, Yang & Kallio, 2014). Within the same ssp. *sinensis*, the Chinese berries from Sichuan had remarkably lower sugar contents compared with wild berries from other growth sites in China, and the content of sucrose in the berries varied widely between different harvesting years (Zheng, Kallio, Linderborg & Yang, 2011, Zheng, Yang, Trepanier & Kallio, 2012).

The presence of inositols and methyl inositols in SB berries has been reported by researchers of our lab (Kallio et al., 2009). L-quebrachitol was the most abundant sugar alcohol in ssp. *sinensis*, methyl-*myo*-inositol the second most abundant, and *myo*-inositol the least (Zheng, Kallio, Linderborg & Yang, 2011). Furthermore, L-quebrachitol was the most distinctive marker for the identification of *H. rhamnoides* ssp. *sinensis* by its NMR fingerprints (Su et al., 2014). The contents of methyl inositols in SB berries were higher in ssp. *sinensis* than in ssp. *rhamnoides* and ssp. *mongolica* (Yang, 2009). As bioactive compounds, inositols can decrease hyperglycemia and hyperlipidemia in diabetic rats and rabbits (Nascimento et al., 2006) and play an important role in insulin secretion of insulin and generation of mediators in diabetic rats (Fonteles, Almeida & Larner, 2000) as well as increase fasting insulin level in plasma in mice (Xue et al., 2015). Further, insulin resistance-related diseases were associated with derangements in inositol metabolism (Muscogiuri, Palomba, Laganà & Orio, 2016).

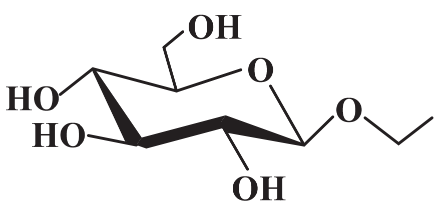


Figure 4. Ethyl *β*-d-glucopyranoside (EG**)**

Ethyl *β*-D-glucopyranoside (EG) is a compound characteristic for sea buckthorn but rarely found in other edible berries or fruits (**Figure 4**). In 2006, the presence of EG was reported in SB for the first time (Tiitinen, Yang, Haraldsson, Jonsdottir & Kallio, 2006). Presence of a corresponding methyl derivative was also reported in sea buckthorn in 2014 (Lindstedt et al., 2014). 'Tytti' and 'Terhi', both cultivars from Kittilä (North Finland) contained very little EG. Whereas they had higher and similar levels of *O*-methyl *β*-D-glucopyranoside (methyl glucoside) over EG in the samples studied (Kortesniemi, Sinkkonen, Yang & Kallio, 2017). It has been reported that peels of yuzu (*Citrus junos Sieb*) also contained EG (Sawabe & Matsubara, 1999).

Notable amounts of EG exist in SB berries of *H. rhamnoides* ssp. *rhamnoides*. However, the content of EG is varied considerably among subspecies and cultivars (Kortesniemi, Sinkkonen, Yang & Kallio, 2017, Yang, 2009, Zheng, Yang, Trepanier & Kallio, 2012). In some berries of ssp. *rhamnoides*, the content of EG dominated in the sugar fraction, and the content of EG was suggested to be among the major chemotaxonomic characteristics in SB berries (Yang, 2009). The content of this compound along with other sugars varied greatly with genetic background, harvesting time, and origin (Ohkawa et al., 2009, Tiitinen et al., 2006, Yang, 2009, Zheng et al., 2011, Zheng et al., 2012). *H. rhamnoides* ssp. *mongolica* and ssp. *sinensis* typically contained only trace amounts of EG, whereas the compound was significantly higher content in the berries of ssp. *rhamnoides* (Yang, 2009). It has been reported that the content of EG in the berries of ssp. *rhamnoides* increases during harvesting period accompanied by a decrease in the content of glucose (Yang, 2009). However, in the berries of cultivar 'Russian Orange' of ssp. *mongolica*, glucose content increased significantly during ripening, whereas the contents of EG, sucrose and fructose remained constant (Ohkawa et al., 2009).

Both ethyl and methyl glucosides have been found in human plasma and urine after a SB meal based on an NMR metabolomics study (Lindstedt et al., 2014). Little information is available on the absorption and metabolism of EG in animals or in humans, although the compound may have some relevance to the physiological effects. Matsubara et al. reported that EG showed a blood pressure decreasing effect in SHR rats after intravenous injection of the compound (Matsubara, Mizuno, Sawabe, Iizuka & Okamoto, 1989). Some researchers have found that EG was transported through the small intestinal wall (Higgins, Miller & Denyer, 1996, Storlien, James, Burleigh, Chisholm & Kraegen, 1986), indicating that EG ingested orally might be absorbed into blood stream. In a recent study, EG was found in its intact form in the urine of rats after oral administration of the compound, and that a small amount remained in the rat body 24 h after administration (Mishima, Harino, Sugita, Nakahara, Suzuki & Hayakawa, 2008). EG exhibited hypotensive effect by examination using stroke-prone spontaneously hypertensive rats (Sawabe & Matsubara, 1999).

Non-phenolic organic acids are widely distributed in fruits and vegetables, such as citric (A), quinic (B), malic (C) and ascorbic acid (D), the structures of which were shown in **Figure 5**. Malic and quinic acids dominated in SB berries, whereas minor levels of citric acid were reported (Tiitinen, Hakala & Kallio, 2005, Zheng, Kallio, Linderborg & Yang, 2011). In a study by Su et al. (2014), the berries of *H. rhamnoides* ssp. *gyantsensis* and *H. tibetana* were separated based on the differences in metabolites between the species by NMR. High content of malic acid and abundance of quinic acid were the most distinctive fingerprints for *H. tibetana* and *H. rhamnoides* ssp. *gyantsensis,* respectively. SB berries of the cultivar 'Terhi' and 'Tytti' from north Finland had relatively higher levels of quinic acid compared with those of the same cultivars grown in Canada (Kortesniemi, Sinkkonen, Yang & Kallio, 2017). Fatima et al. reported that ascorbic acid was synthesised from guanosine diphosphate (GDP)-d-mannose in SB berry, *via* the L-galactose or the L-glucose pathway (Fatima et al., 2015). The content of ascorbic acid in SB leaves is high (Hellström, Pihlava, Marnila, Mattila & Kauppinen, 2013). Its content in berry was found to be mainly determined by the genotype and time of harvest (Kallio, Yang & Peippo, 2002), and differences in content occurred also between cultivars of the same subspecies (Hussain, Ali, Awan, Hussain & Hussain, 2014, Kalinina, Panteleyeva & Kryukov, 1987).

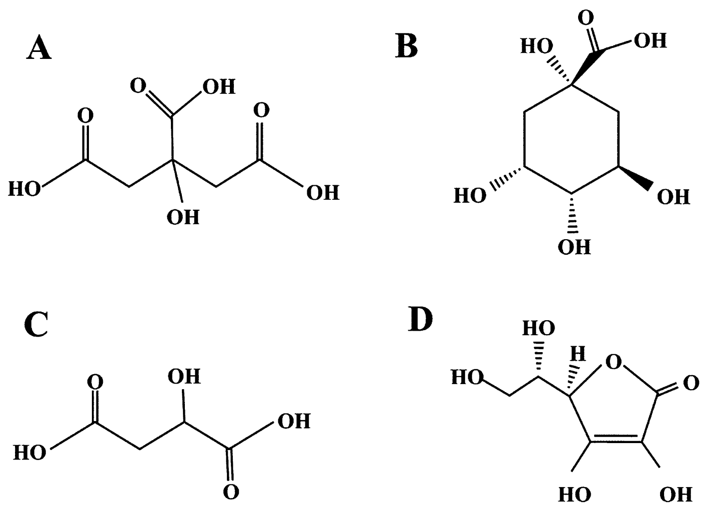


Figure 5. Examples of non-phenolic organic acids in se buckthorn berry: citric acid (A), quinic acid (B), malic acid (C) and ascorbic acid (D).

Differing from the pattern of variation of sugars, a marked decline in the concentrations of total organic acid and ascorbic acid was observed during ripening of SB berries in the 'Askola', 'Hergo' and 'Leikora' of German cultivars (Raffo, Paoletti & Antonelli, 2004). Various weather conditions, such as temperature, light, water supply and air humidity are important factors affecting the accumulation of sugars and acids in SB (Buchanan, Gruissem & Jones, 2000, Kortesniemi, Sinkkonen, Yang & Kallio, 2017, Zheng, Kallio, Linderborg & Yang, 2011, Zheng, Yang, Trepanier & Kallio, 2012). For example, sea buckthorn berries of ssp. *mongolica* grown at lower latitude had higher levels of total sugar and lower levels of total acids than those grown at higher latitude (Zheng, Yang, Trepanier & Kallio, 2012). In wild Chinese sea buckthorn (ssp. *sinensis*), the contents of fructose, glucose, and total sugars decreased, whereas the contents of malic acid and ascorbic acid increased, as the altitude increased and as the latitude decreased (Zheng et al., 2011).

2.2 Contribution of various metabolites to sensory properties of sea buckthorn

Sensory characteristics are the most important drivers of consumer preferences and choices within each food category (Geertsen et al., 2016). SB berries are known to be quite sour, bitter, and astringent, but not very sweet, with a mild but characteristic aroma and taste, not comparable to any other berries or fruits. Due to these characteristics, SB berries and berry products are not commonly used as ingredients in food industry. It is essential to investigate the contribution of various compounds to the sensory profile of SB berries. The knowledge is needed for breeding and cultivation as well as industrial processing of SB.

Although volatile compounds will definitely influence the flavor of SB berry and berry products, we did not include them in this review, which is focused on the role of non-volatile compounds important for sensory and bioactive properties of sea buckthorn.

2.2.1 Sourness and sweetness

Sourness and sweetness are two of the five basic taste qualities of food. Sourness is perceived through specific channels of PKD2L1 located in taste cells, and sour taste is mediated by a unique cell type, independent of all other taste qualities (Huang et al., 2006). Intense sourness can evoke an innate rejection response in adult humans and many other animals, whereas sourness and acidity of low levels may be perceived as an attractive flavor in food (Kim, Breslin, Reed & Drayna, 2004).

Organic acids, such as malic and citric acids, are typically main acids contributing to the sourness of berries and fruits (Laaksonen, Sandell & Kallio, 2010, Sandell et al., 2009, Tiitinen, Hakala & Kallio, 2005). Malic acid was the major acid contributing to the sourness of SB berries. Quinic and citric acids also contributed to sourness; however, the former was also closely associated with astringency and bitterness of SB berries (Ma, Laaksonen, Heinonen, Sainio, Kallio & Yang, 2017). The intensity of sourness was positively correlated with astringency, negatively, with sweetness of SB juices (Tang, Kälviäinen & Tuorila, 2001, Tiitinen, Hakala & Kallio, 2005). The decrease in the acid contents also had the evident impact on the flavor mainly *via* the reduction of sourness (Tiitinen, Vahvaselkä, Hakala, Laakso & Kallio, 2006).

SB berries were perceived with low intensity of sweetness (Tang, Kälviäinen & Tuorila, 2001, Tiitinen, Hakala & Kallio, 2005). Glucose and fructose were the major sugars, which contributed to the sweetness of the SB berries (Tang, Kälviäinen & Tuorila, 2001, Tiitinen, Hakala & Kallio, 2005, Yang, 2009). The consumer rating of sweetness intensity was the primary factor contributing to overall liking, thus sweetness had strong positive effects on SB juice pleasantness (Tang, Kälviäinen & Tuorila, 2001). The contents of sugars and acids, especially the sugar/acid ratio, played a key role in determining the flavor and consumer acceptability of berries and berry products (Laaksonen et al., 2013, Tang, Kälviäinen & Tuorila, 2001, Tiitinen, Hakala & Kallio, 2005)

2.2.2 Astringency

The oral sensation of astringency is commonly described as a long-lasting trigeminal sensation in the oral cavity. It can be classified into several sub-qualities, such as velvety, grainy, drying, or puckering (Gawel, Iland & Francis, 2001). Astringency is an important sensory character of SB berries. Phenolic compounds were considered as the major compounds responsible for astringency of SB (Ma, Guo, Zhang, Wang, Liu & Li, 2014, Soares, Brandão, Mateus & De Freitas, 2017). Sea buckthorn berries are a rich source of FGs (Ma et al., 2016, Teleszko, Wojdyło, Rudzińska, Oszmiański & Golis, 2015, Yang, Halttunen, Raimo, Price & Kallio, 2009) and they also contain notable amounts of proanthocyanidins (PC and PD), up to 250 mg/100 g. (Hellström, Törrönen & Mattila, 2009, Rösch, Mügge, Fogliano & Kroh, 2004, Yang, Laaksonen, Kallio & Yang, 2016b).

Flavonol glycosides have been found to induce a silky, mouth-drying, and mouth-coating astringent sensation. In comparison to the condensed tannins, FGs have been reported to have a more important role in astringency of wine (Hufnagel & Hofmann, 2008a). Quercetin-3-*O*-rutinoside was reported to be one important key compound in astringency of red currant (*Ribes rubrum*) and black tea (Scharbert, Holzmann & Hofmann, 2004, Schwarz & Hofmann, 2007). Among the FGs, this compound can elicit astringent properties at the lowest concentration, 0.001 µmol/L in bottled water, which was far below those of catechins (410 µmol/L) or theaflavins (16 µmol/L) (Scharbert, Holzmann & Hofmann, 2004, Schwarz & Hofmann, 2007). Quercetin-3-*O*-rutinoside was found in significant amounts in sea buckthorn berries. In the berries of ssp.

sinensis, the content of this compound was on average 10 mg/100 g FW, which was much higher than that of ssp. *mongolica* (3.8 mg/100 g FW) (Ma et al., 2016). Based on this it might be predicted that the berries of ssp. *sinensis* may taste more astringent than those of ssp. *mongolica.* However, quercetin glycosides had less impact on the astringency of SB purees of 6 cultivars studied ('Terhi', 'Tytti', 'Hergo', 'Leikora', 'Trofimovskaya' and 'Avgustinka') (Ma et al., 2017). Isorhamnetin was the predominant aglycone moieties of FGs in SB berries. Although their sensory thresholds for astringency have been not reported, isorhamnetin glycosides, especially isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside, were found to be closely associated with the astringent attributes in the SB purees (Ma et al., 2017).

Proanthocyanidins were perceived more puckering and rough than FGs. The key astringent compounds in wine have been reported to be various PAs, of which oligomers are more astringent than the monomers (Hufnagel & Hofmann, 2008a). In sea buckthorn berries, the PA dimers and trimers have been detected by Kallio et al. (Kallio, Yang, Liu & Yang, 2014). The PA dimers and trimers are important components contributing to bitterness and astringency of red wine, and molecular size was the major factor influencing the sensory properties of bitterness and astringency (Hufnagel & Hofmann, 2008a). However, the known astringent compounds, PA oligomers, showed less impact on the astringency than FGs in SB purees of cultivars 'Terhi', 'Tytti', 'Hergo', 'Leikora', 'Trofimovskaya' and 'Avgustinka' (Ma et al., 2017).

The astringent sensory hresholds of procyanidin B1, B2 and B3 were reported to be 240, 190 and 200 μmol/L in water, respectively (Hufnagel & Hofmann, 2008a). The astringent threshold decreased from the PA monomers (+)-catechin and (−)-epicatechin over the dimeric procyanidins B1, B2, and B3, to the trimeric procyanidin C1. The content of PAs and the ratio of PC/PD effect on the mouth-drying and puckering astringent characteristics in black currant juices, higher PC/PD ratio being associated with decreased astringency (Laaksonen, Salminen, Mäkilä, Kallio & Yang, 2015).

Procyanidins are perceived as more astringent than ellagitannins (ETs), but the latter have lower thresholds for detection of astringency spanning from 0.2 to 6.3 μmol/L (Chira & Teissedre, 2013, Hofmann, Glabasnia, Schwarz, Wisman, Gangwer & Hagerman, 2006). Sea buckthorn leaves contain abundant ETs (Moilanen, Koskinen & Salminen, 2015, Suvanto et al., 2018), but the role of ETs in the sensory quality in SB leaves products has not been investigated.

In addition to the phenolic compounds described above, various phenolic acid derivatives and organic acids have been reported to elicit astringent sensations and have astringent properties (Hufnagel & Hofmann, 2008a, Lawless, Horne & Giasi, 1996, Scharbert, Holzmann & Hofmann, 2004). Puckering astringency was found to correlate positively with sourness contributed by malic acid, quinic acid, ascorbic acid and the total acid content in SB juices (Ma et al., 2017). The level of pH had the major influence on astringency of sensation (Lawless, Horne & Giasi, 1996), and an increase in astringency was often associated with a decrease in pH (Peleg & Noble, 1999). The acidity of wine can indirectly contribute to astringency by affecting the efficacy of bonding of polyphenols to salivary proteins (Perez-Maldonado, Norton & Kerven, 1995). Food matrix plays an important role in the astringency perceived. For instance, some astringent reference substances, such as tannic acid and alum, tasted more astringent in water than in wine or orange juice (Valentová, Skrovánková, Panovská & Pokorný, 2002).

2.2.3 Bitterness

Bitterness is a major sensory property of foods and beverages rich in polyphenol compounds. Due to the wide variety of compounds that elicit bitterness and to the apparently large number of genes encoding receptors for this taste modality, the sensation of bitterness also appears to be the most complex taste quality in humans. Bitter tastes are sensed through the binding of the tastants to G-protein-coupled type 2 receptors (TAS2R), which locate within the papillae of the tongue. There are 25 TAS2R receptors involved in bitter perception (Meyerhof et al., 2010). *Beta*-D-glucopyranosides have been found to elicit bitterness through the binding of TAS2R16 (Bufe, Hofmann, Krautwurst, Raguse, & Meyerhof, 2002; Sakurai et al., 2010). It has been reported that bitterness of the SB juices related to the EG content as well as the ratios of EG/acids and EG/sugars (Ma et al., 2017, Yang, 2009). Interestingly, sensory properties of ethyl *α*-D-glucopyranoside were also investigated in sake, and a sweet taste with a bitter aftertaste was reported (Yabiku et al., 2016). This indicated that ethyl *α*-D-glucopyranoside activates not only sweet receptors but also bitter receptors.

Various phenolic compounds are also related to bitter properties. In a recent study, various monomeric flavonoids, including (+)-catechin, (−)-epicatechin, and (−)-epigallocatechin, activated the human bitter taste receptors TAS2R14 and TAS2R39 (Roland et al., 2013). Both bitter and astringent compounds, such as (+)-catechin and (−)-epicatechin, can be found in one food (red wine or black tea infusions). Generally, most of the phenolic compounds can be perceived as astringent at notably low concentrations and as bitter at high concentrations. The taste threshold concentration of (+)-catechin for bitterness was 290 mg/L, whereas its astringent threshold concentration was 119 mg/L in water by triangle test (Hufnagel & Hofmann, 2008a).

ETs have an impact on bitterness sensation at a higher concentration than astringency. For example, castalagin and vescalagin, identified in SB leaves, their bitter recognition thresholds were both 1578 mg/L, which was the 1500 times higher than astringent threshold (Glabasnia & Hofmann, 2006, Hofmann, Glabasnia, Schwarz, Wisman, Gangwer & Hagerman, 2006). Some studies have shown that bitterness of polyphenols increases with the molecular weight (Arnold, Noble & Singleton, 1980, Hufnagel & Hofmann, 2008b). Hufnagel and Hofmann found that procyanidin dimers and trimers were more bitter than (−)-epicatechin (Hufnagel & Hofmann, 2008b). Additionally, some non-bitter phenolic astringent compounds such as quercetin-3-*O*-rutinoside, may enhance the intensity of the perceived bitterness of caffeine (Scharbert & Hofmann, 2005).

The contents of total PAs and PA oligomer did not contribute to the bitter taste in SB purees (Ma, Yang, Laaksonen, Nylander, Kallio & Yang, 2017). However, the ratios between acids and various phenolic compounds, such as PAs and FGs, were shown to be strongly associated with bitterness in SB (Ma, Yang, Laaksonen, Nylander, Kallio & Yang, 2017). These findings also indicate the existence of other bitter compounds in SB in addition to PAs.

Although many phenolic compounds have been shown to activate bitter taste receptors, the bitterness perception by humans depends on the taste thresholds of the bitter compounds and the food matrix. Sensory thresholds for some compounds studied in this thesis have been reported in previous studies. The taste threshold concentrations of B-type PAs for bitterness are in the range of 231−289 mg/L, as determined in water by triangle tests (Hufnagel & Hofmann, 2008a). ETs were perceived as bitter with threshold concentrations between 87 and 1578 mg/L in water (Glabasnia & Hofmann, 2006). The taste threshold of pure EG was 1.1 ± 1.3 g/L for bitterness, according to an assessment carried out with water solution by Duo-Trio tests (Ma, Laaksonen, Heinonen, Sainio, Kallio & Yang, 2017). However, there is the existence of extremely complex interactions among non-volatile compounds on the in-mouth sensory perception. The sensory thresholds vary with different food matrices and among human subjects. Additionally, sea buckthorn contains quite many phenolic compounds, the thresholds of which have not been reported. Investigations of the taste thresholds of these compounds would require lengthy isolation and purification process, because many of the compounds are not commercially available as pure references.

2.3 Challenges in sea buckthorn products development

Sea buckthorn (*Hippophaë rhamnoides* L.), an ancient plant, has recently sparkled significant interest, due to the nutritional and medicinal value (Bal, Meda, Naik & Satya, 2011, Rousi, 1971). All parts of SB including berries, leaves, seeds, and bark are rich sources of bioactive compounds (Guo, Guo, Li, Fu & Liu, 2017, Hellström, Pihlava, Marnila, Mattila & Kauppinen, 2013, Kallio, Yang, Peippo, Tahvonen & Pan, 2002b, Korekar, Stobdan, Singh, Chaurasia & Singh, 2011). Human studies as summarized by Christaki suggest that SB may have various beneficial effects such as cardio-protective, antioxidant, anti-cancer, immunomodulatory, anti-bacterial, antiviral, wound-healing and anti-inflammatory properties (Christaki, 2012). Sea buckthorn could also be used to improve the nutrition of humans and animals. Therefore, it is worthwhile to develop more products based on different parts of this plant and to promote its large-scale utilization. The quality of SB products depends on the quality of raw materials and processing technologies. Furthermore, special challenges in food product development based on sea buckthorn come from the low sweetness and strong taste of sourness because of the low sugar content, high acid level, as well as astringency and bitterness due to the presence of various phenolic compounds.

2.3.1 Quality of raw material

Cultivation practices affect the content of bioactive compounds, such as flavonols, phenolic acids, tannins, lipids, and Vitamin C in SB berries and leaves (Häkkinen & Törrönen, 2000, Heinäaho, Pusenius & Julkunen-Tiitto, 2006, Heinäaho, Hagerman & Julkunen-Tiitto, 2009). The influences of different organic cultivation methods on the berry and leaf phenolics of two sea buckthorn (ssp. *rhamnoides*) cultivars, ‘Terhi’ and ‘Tytti’, were investigated in an experimental field of Finland. In the study of SB berry, flat land increased the amounts of isorhamnetin and quercetin compounds, as well as condensed tannins (Heinäaho, Hagerman & Julkunen-Tiitto, 2009). The use of plastic mulch decreased the concentrations of ETs and condensed tannins in SB leaves compared to the other mulches used (Heinäaho, Pusenius & Julkunen-Tiitto, 2006). These results indicate that the phenolic accumulation in berries and leaves of SB cultivars ‘Terhi’ and ‘Tytti’ seems to be mainly dependent upon soil structure. In addition, the material of mulch is also an important factor influencing bioactive compounds, especially tannins in the leaves.

The temperature of storage of SB juice is very important. Storage at room temperature (25 °C) may result in a significant degradation of pantothenic acid (Gutzeit, Klaubert, Rychlik, Winterhalter & Jerz, 2007a). SB juice turns brown after about 6 months of storage at 15–20 °C (Liu & Liu, 1989). Storage also often leads to changes in phenolic compounds due to temperature, light and enzymes. Flavonol glycosides stayed fairly stable comparing with anthocyanins (Makila et al., 2016). Enzymes and sunlight are important initiators of browning (Zhou & Chen, 1989). It is not clear if the brown color develops as a result of residual enzyme, such as residual polyphenol oxidase, or if the brown color develops because of nonenzymatic (Maillard or vitamin C) browning.

2.3.2 Processing of sea buckthorn products

During the past years, SB has gained recognition worldwide. SB berry products are becoming increasingly popular as health foods in many countries in Asia, Europe, and North America. Sea buckthorn berries are commonly processed into various products such as juices, jams, as well as seed oil and pulp oil. Processing may induce changes in texture and flavor, thus affect the quality of SB products.

Processing of juice from SB berries is a complex operation with many variables that influence the final product quality. Freshly pressed SB juice separates into three phases when left standing for a short period at refrigerating temperatures (0−10 °C): an upper creamy phase, a central juice portion, and a sediment at the bottom (Beveridge, Harrison & Drover, 2002). Separation is undesirable in juice products from the consumers' perspective (Kleinschmidt, Siudzinski & Lange, 1996). Appropriate processing technologies and conditions need to be applied in order to maintain the stability of the juice as well as the nutritional and sensory characteristics. Centrifugation is used to separate the oil and to reduce the oil content in the juice to levels of less than 0.1%, which will eliminate the problem of having oil layer on the surface of the juices. Commercially available hydrolytic enzymes, such as pectinmethylesterase (PME), may be used to improve the juice yield, which also affect the viscosity and cloud stability of the juice (Espachs-Barroso, Van Loey, Hendrickx & Martín-Belloso, 2006). Appropriate soaking of berries before juice pressing was shown to reduce soluble solids (°Brix) in juice because of uptake of water (Beveridge, Harrison & Drover, 2002).

Conventional thermal processing is used for assuring safety and extending the shelf life of foods. But it often causes undesirable detrimental changes in quality indices and sensory characteristics. High-temperature-short-time (HTST) processes at 80–90 °C for several seconds are commonly used (Liu & Liu, 1989). SB juices are somewhat delicate and will suffer from a loss of flavor and develop an off-flavor if heated beyond the conditions indicated. Moreover, during industrial juice production, the HTST processing of the SB berries caused a loss of about 5% to 11% total ascorbic acid in the juice (Gutzeit, Baleanu, Winterhalter & Jerz, 2008). Production of the juice concentrate by thermal-vacuum evaporation at 80−85 °C, resulted in 50% depletion of ascorbic acid (Gutzeit, Baleanu, Winterhalter & Jerz, 2008). Thus, the use of non-thermal technologies is gaining popularity. High pressure (HP) processing is a mainstream non-thermal process, specifically, the process of high pressure (200−600 MPa) cold pasteurization (< 35 °C) can be used for commercial production of superior quality SB juices to meet the demand of consumers for high quality product (Alexandrakis et al., 2014).

The most recognizable SB product is oil pressed or extracted from the pulps, especially due to the presence of palmitoleic acid, which is very uncommon in the plant kingdom (Fatima et al., 2012, Yang & Kallio, 2002). The efficiency of pulp oil recovery varies widely with the design of the process. The usual methods for manufacturing oil commercially require countercurrent extraction of the oil bearing material, seed or pulp, with an organic solvent, commonly hexane (Li, Beveridge & Drover, 2007b). However, solvent extraction is not recommended for high value ingredients meant for nutraceutical applications due to the residual solvents and the destruction of bioactive phytochemicals during removal of the solvent. Recently, supercritical carbon dioxide (SC-CO_2) extraction has attracted considerable attention for solvent-free oil of superior quality and for extraction of aroma compounds (Herrero, Cifuentes & Ibañez, 2006, Šťastová, Jeẑ, Bartlova & Sovová, 1996). SC-CO_2 has been widely used for extracting oils from both seeds and pulp of SB berries (Yakimishen, Cenkowski & Muir, 2005). The extraction gives a higher content of phytosterol in seed oil than cold pressing or extraction with hexane (Li, Beveridge & Drover, 2007a, Sajfrtová, Ličková, Wimmerová, Sovová & Wimmer, 2010). However, the berries are often dried before extraction to obtain reasonable efficiency of extraction, which will result in a loss of phytonutrients during drying.

Although the processing methods of sea buckthorn are widely established through numerous studies, limited information is available about how these bioactive components and their bioactivities are affected by different processing methods. The flavonoids varied significantly among the different thermal processing (50–100 °C), whereas the processing had less effect on the carotenoids and polyphenols in SB extracts (Ursache, Ghinea, Turturică, Aprodu, Râpeanu & Stănciuc, 2017). ET concentrations of some products (berries juices and wines) increased after processing, whereas some ETs degraded to ellagic acids (Bakkalbaşi et al., 2008). During the processing of black currant juice, FGs seemed to be more stable than other phenolic compounds (Mäkilä, Laaksonen, Kallio & Yang, 2017). Along with changes in phenolic compounds, processing conditions have an important effect on antioxidant activity (Bakkalbaşi et al., 2008, Donlao & Ogawa, 2018, Kyriakopoulou et al., 2013). Freeze-drying provided higher radical scavenging ability of SB berries' extracts, whereas, the extract obtained by the microwave extraction of fresh berries exhibited lower antioxidant activity compared to the ones of freeze-dried berries (Kyriakopoulou, Pappa, Krokida, Detsi & Kefalas, 2013). Additionally, the drying process can cause changes in texture, shape, and color of sea buckthorn products. Freeze-drying had a better appearance of berries' extracts (Kyriakopoulou et al., 2013). For pulp oil of sea buckthorn, air-drying gave a significant higher extraction yields compared with freeze-drying (35.9 ± 0.8 vs 17.1 ± 0.6% w/w) (Gutiérrez, Ratti & Belkacemi, 2008).

In addition to berries, leaves of SB contain a variety of nutrients and bioactive substances (Hellström, Pihlava, Marnila, Mattila & Kauppinen, 2013, Pop, Weesepoel, Socaciu, Pintea, Vincken & Gruppen, 2014). Compared with berries, SB leaves have the advantages of high yield, easy collection, long acquisition cycle, simple production and processing, easy storage. Numerous products can be made from the dried leaves such as tea-type beverages and tea-type powder products. Drying process can cause changes in the texture, shape, and color of SB leaves (Kyriakopoulou et al., 2013). Air-drying has resulted in the better appearance in the leaf extracts, while freeze-drying has provided more stable results than air- or oven-drying in birch (*Betula pubescens*) leaves, due to the lower temperatures at beginning of the drying process (Kyriakopoulou et al., 2013, Salminen, 2003). Moreover, SB leaves have been reported to retain considerable amounts of total phenolics and carotenoids comparable to those of commonly consumed vegetables during drying at an elevated temperature (Guan, Cenkowski & Hydamaka, 2005). However, drying process of leaves definitely resulted in a decrease in the concentrations of phytochemicals. The degree of reduction depends on the drying time, temperature, or specific type of compounds (Guan, Cenkowski & Hydamaka, 2005). Thus, processing methods should be optimized in order to maximally obtain a specific bioactive compound.

Another potentially large application for SB, are products for animal nutrition. The large volume of “waste” material, such as leaves and bark, as well as residues from juice and oil extraction, could be developed into value-added products, since the “waste” materials still contain valuable nutrients and bioactive compounds (Hellström, Pihlava, Marnila, Mattila & Kauppinen, 2013, Mäkilä et al., 2014, Puganen, Kallio, Schaich, Suomela & Yang, 2018).

2.3.3 Food development and consumer acceptance

Product development is essential for industry to grow and to be competitive in the global market. It is increasingly important to be consumer orientated in product development. Sensory properties of food are the key factors for food acceptance by consumers.

SB berries are a good choice as healthy foods containing a wide range of bioactive compounds, among which are various phenolic compounds. However, these phenolics also bring challenges due to a negative impact on perceived sensory quality, such as astringency and bitterness (Laaksonen, Knaapila, Niva, Deegan & Sandell, 2016). These sensory characteristics can be generally considered as negative factors in the acceptance of berry or berry products (Lesschaeve & Noble, 2005). Despite of these possible health effects, consumers may not be willing to choose healthiness over a good taste (Verbeke, 2006). On the other hand, consumers sometimes prefer complex and balanced tastes, and enjoy astringency and bitterness in some specific types of beverages, such as coffee, tea, beer and wine (Geertsen, Allesen-Holm, Byrne & Giacalone, 2016). Certain degree of complexity in taste may enhance consumer acceptance of fruit juices.

Chemical constituents of berries have a strong influence on the sensory quality of the berries thus affecting the consumer acceptance of berry and berry products. Besides the phenolic compounds, various organic acids, sugars, EG and the ratios of sugar/acid, EG/sugars, EG/acids as well as phenolic compounds/acids contribute to the sensory quality of berries and have an important influence on the pleasantness (Laaksonen, Knaapila, Niva, Deegan & Sandell, 2016, Ma, Yang, Laaksonen, Nylander, Kallio & Yang, 2017, Ma, Laaksonen, Heinonen, Sainio, Kallio & Yang, 2017). Additionally, growth sites, harvest time, storage or packaging type used for berry and berry products may affect the quality, thus influencing the consumer acceptance (Almenar, Samsudin, Auras & Harte, 2010, Gutzeit, Klaubert, Rychlik, Winterhalter & Jerz, 2007b, Ma et al., 2016, Ohkawa, Kanayama, Chiba, Tiitinen & Kanahama, 2009).

Berries of SB have a unique aroma and flavor, but perceived as strongly sour and astringent mainly due to the high acid content (Tiitinen, Hakala & Kallio, 2005, Tiitinen, Yang, Haraldsson, Jonsdottir & Kallio, 2006). Hence, pure SB juice (average pH 3.13) is characterized by high acidity and astringency. From a sensory point of view, oral sensations need to be balanced to reach likeable products (Beveridge, Li, Oomah & Smith, 1999, Laaksonen, Knaapila, Niva, Deegan & Sandell, 2016). Thus, some sweeter fruits were used to balance the sourness and astringency of SB to produce mixed juices in the development of SB-based products (Selvamuthukumaran, Khanum & Bawa, 2007). Recently, a multi-fruit jelly was developed by blending SB juice with papaya, watermelon or grapes in varying proportions. The malolactic fermentation (MLF), traditionally used in winemaking, was applied also to reduce the high sourness of SB juice (Tiitinen, Vahvaselkä, Hakala, Laakso & Kallio, 2006).

Besides the sensory quality of food, consumer acceptance of food products is also influenced by extrinsic properties, such as health claims, life habit, and price of products, appearance of package and the labelling as well as brand. Furthermore, social factors, such as expert recommendations, brand familiarity and expectations of consumers for the product have the influence on the hedonic response (Saba, Moneta, Nardo & Sinesio, 1998).

2.4 Conclusions and future prospects

Sea buckthorn is a valuable new field crop currently being a target of interest all over the world. The berries and leaves are good sources of valuable compounds with nutritional and therapeutic effects. These beneficial effects make SB products increasingly known. Although sea buckthorn industry has yet to be developed to a larger scale and the sensory properties limit the consumption of fresh berries to some extent, sea buckthorn products may play an important role in the food, nutraceutical and cosmetic market in the future.

3 AIMS OF THE STUDY

The research was focused on the investigation of structures and quantities of selected sea buckthorn (SB) components and their effects on the quality of SB berries and leaves as raw materials of food, beverage and food ingredients.

The specific aims were:

1. To study the composition and content of flavonol glycosides (FGs) in berries and berry purees, and in tea-type beverages produced from SB leaves (papers **I, III** and **IV**).
2. To investigate the effect of genetic background (berries of subspecies *sinensis*, *mongolica* and *rhamnoides*; berries and leaves of cultivars), altitude and latitude of growth location, and drying process of leaves on the content and composition of FGs (papers **I** and **IV**).
3. To study the contribution of FGs, proanthocyanins (PAs) and ethyl *β*-D-glucopyranoside (EG) to the sensory qualities of sea buckthorn purees/juices (papers **II** and **III**).
4. To investigate the antioxidant activities (AAs) in the tea-type beverages prepared from SB leaves, and correlations between FGs, ETs and AAs (paper **IV**).

4 MATERIALS AND METHODS

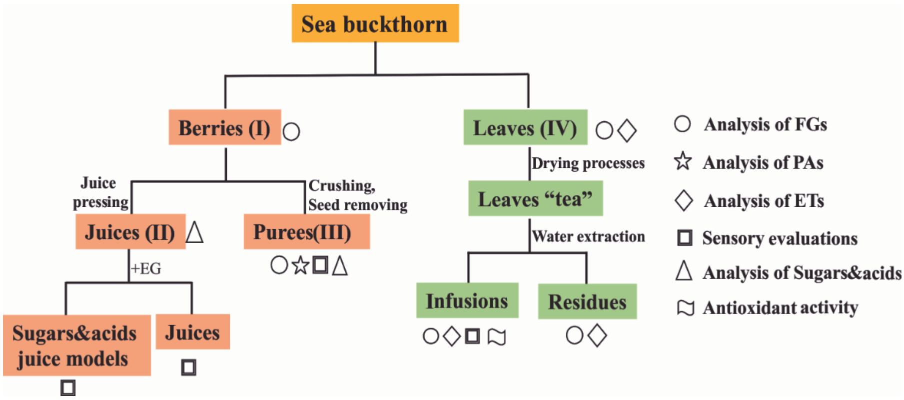


Figure 6. Overall scheme of sample preparations and analyses conducted in this thesis.

4.1 Research material

4.1.1 Sea buckthorn berries and leaves

The sea buckthorn berries and leaves used in this work are summarized in **Table 2**. Wild and cultivated berries of three subspecies and leaves of two cultivars of SB were investigated in the thesis. Berries of *H. rhamnoides* ssp. *mongolica* were collected from four growth sites in Finland, Canada and Estonia inconsecutively during a period of twelve years. Wild berries of *H. rhamnoides* ssp. *sinensis* were collected from nine natural growth-sites in six provinces in China from 2006 to 2008. Berries of 'Terhi' and 'Tytti' of ssp. *rhamnoides* were harvested in August and October in 2014 and 2015 in Turku in southwestern Finland. The berries of SB cultivars 'Hergo', 'Leikora', 'Trofimovskaya' and 'Avgustinka' were collected in Röhu experimental station in Estonia in September 2015. In each growth place, the berries were picked randomly from different blocks as optimally ripe. All the berries were frozen immediately after picking and stored at −18 °C until analysis. Sealed boxes were used to keep the shape and quality of berry. In the long storage of samples, it is not excluded that some berries would dry or some degradations of the compounds happened in the berries.

Flavonols of berries from Finland, Canada and China were studied in paper **I**. The role of ethyl β-D-glucopyranoside (EG) in sensory quality of berries from two typical Finnish SB cultivars 'Terhi' and 'Tytti' were investigated in paper **II** and the contribution of flavonols and proanthocyanins (PAs) to the sensory qualities of berries from Finland and Estonia were analyzed in paper **III.**

Leaves of two sea buckthorn cultivars, ‘Terhi’ and ‘Tytti’, were harvested in August 2015 in Turku, Finland (**IV**). The leaf samples were picked from random sites in 4−6 bushes and mixed for each cultivar. The samples were stored at −18 °C after picking until analysis.

4.1.2 Ethyl *β*-D-glucopyranoside

Ethyl *β*-D-glucopyranoside (EG) was provided by the Lappeenranta University of Technology in Finland. The purity of EG was verified with HPLC analysis to be over 98 %.

Table 2. Sea buckthorn berries and leaves investigated in the thesis.

Study	Subspecies	Cultivar	Growth site	Longitude	Latitude	Altitude(m)	Harvest year
Sea buckthorn berries							
I	Ssp. *mongolica*	'Avgustinka' (AVG)	Turku, Finland (TU)	22°09′ E	60°23′ N	1	2003–2006
			Kittilä, Finland (KI)	24°37′ E	68°02′ N	210	2003
		'Botanicheskaya' (BOT)	Turku, Finland (TU)	22°09′ E	60°23′ N	1	2002–2004; 2006; 2008; 2011–2012
			Kittilä, Finland (KI)	24°37′ E	68°02′ N	210	2003
		'Trofimovskaja' (TRO)	Turku, Finland (TU)	22°09′ E	60°23′ N	1	2003; 2004; 2006; 2008; 2011–2012
			Kittilä, Finland (KI)	24°37′ E	68°02′ N	210	2002–2003
		'Pertsik' (PER)	Turku, Finland (TU)	22°09′ E	60°23′ N	1	2003–2004; 2006
			Kittilä, Finland (KI)	24°37′ E	68°02′ N	210	2003
		'Prevoshodnaya' (PRE)	Kittilä, Finland (KI)	24°37′ E	68°02′ N	210	2003
			Québec city, Canada (QU)	71°17′ W	46°47′ N	100	2007– 2010
		'Prozcharachnaya' (PRO)	Turku, Finland (TU)	22°09′ E	60°23′ N	1	2003–2004; 2006; 2008
			Kittilä, Finland (KI)	24°37′ E	68°02′ N	210	2003
			Québec city, Canada (QU)	71°17′ W	46°47′ N	100	2007–2010
		'Chuiskaya' (CHU)	Québec city, Canada (QU)	71°17′ W	46°47′ N	100	2007–2010
		'Oranzhevaya' (ORA)	Québec city, Canada (QU)	71°17′ W	46°47′ N	100	2007–2010
		'Vitaminaya' (VIT)	Québec city, Canada (QU)	71°17′ W	46°47′ N	100	2007–2010
	Ssp. *sinensis*	Wild	Heilongjiang, China (HLJ)	127º06′ E	47º14′ N	210	2006–2008
			Hebei, China (HB)	116º34′E	41º17′ N	818	2006–2008
			Qinghai, China (QH)	101º23′E	36º45′ N	3115	2006–2008
			Inner Mongolia, China (IM)	109º48′E	39º47′ N	1480	2006–2008
			Shanxi , China (SX)	113º52′E	37º05′ N	1512; 2182	2006–2008
			Sichuan, China (SC)	106º54′E	31º01′ N	2000; 2500; 3000	2006–2008
II	Ssp. *rhamnoides*	'Terhi'	Turku, Finland	22°09′ E	60°23′ N	1	Aug, 2014; Oct, 2014
		'Tytti'					Aug, 2015; Oct, 2015
		'Terhi'					Aug, 2015
		'Tytti'					Aug, 2015
III	Ssp.*mongolica*	'Hergo'	Röhu, Estonia				Sep, 2015
		'Leikora'					
		'Trofimovskaya'					
		'Avgustinka'					
Sea buckthorn leaves							
IV	Ssp. *rhamnoides*	'Terhi'	Turku, Finland	22°09′ E	60°23′ N	1	Aug, 2015
		'Tytti'					

4.2 Sample preparation

4.2.1 Preparation of sea buckthorn juice and puree

The juices were cold-pressed using the method described by Tiitinen et al. (2005). Briefly, SB berries (about 200 g) were thawed in a microwave oven (Whirlpool MW 201, Fonthill Industrial estate, Dublin, Ireland) and homogenized in a Bamix blender (Bamix, Mettlen TG, Switzerland). An HP 2 H Tincture press (Fischer Hafico, Germany) was used to press the mash at a pressure of 19.6 MPa. The juice was stored at 4°C (**II**).

SB berries (about 200 g) were half-thawed in a microwave oven, and then the seeds were removed manually. The seedless fraction of berries was homogenized to puree with the Bamix blender. The puree was divided into two parts; one part was stored at 4°C overnight for sensory evaluations and the other at −18 °C for chemical analysis later (**III**).

4.2.2 Processing of sea buckthorn leaves and tea-type infusions (IV)

Sea buckthorn leaves were processed with five different drying methods (**Table 3**). For each drying method, about 20 g of fresh leaves were used. Prior to infusion, each of the processed SB leaf batches was ground into powder, passed through a 14-mesh sieve and packed in a commercial tea bag. Duplicate infusions were prepared according to a "one-cup-serving" strength by infusing 1.0 g of leaves for 5 min with 100 mL of freshly boiled, carbon filtered water, without agitation, then filtered through Whatman filter paper (Grade 0858, Whatman International, Ltd., Maidstone, U.K.), and cooled to room temperature.

Table 3. The processing method of sea buckthorn leaf "tea".

Processing Method	Method Descriptions
Freeze-drying (FD)	lyophilization for two days at 0.288 mbar and – 40 °C in a freeze-dryer
Steam+High Temperature (S+HT)	"fixing" by steam at 95−100 °C for 30−40 s and then drying by high temperature heating 80−90 °C for 2.5 h
Steam+Different Temperature (S+DT)	steaming as above and drying in four heating steps at 70−80 °C for 35−40 min, at 60−70 °C for 30−40 min, at 80−90 °C for 15−20 min, and at 60−75 °C for 30−40 min
Low Temperature(LT)	drying by low temperature heating 60−70 °C for 3.5 h
Air-drying (AD)	air-drying for three days in the laboratory at ambient temperature of 25 ± 2 °C and relative humidity of 27%

4.2.3 Extraction of flavonol glycosides and ellagitannins

Extractions of FGs of SB berries (**I**) and purees (**III**) were carried out with methanol. The berry slurry/puree of about 8 g was weighted accurately and extracted three times consecutively with 15 mL of methanol. During each extraction, the extraction mixture was thoroughly mixed and centrifuged, and the three clarified extracts were combined, methanol removed with a rotary evaporator. The sample was re-dissolved in 3 mL of methanol and filtered.

Extraction of FGs and ETs from SB fresh leaves, from tea-type infusions, and from infused residues were carried out in duplicate (**IV**). For FG analyses, 20 mL sample of "tea" infusion was freeze-dried, and the freeze-dried powder was dissolved in 2 mL of methanol and filtered. For ET analyses, tea infusions were centrifuged and the supernatants were filtered.

The "tea" residues were extracted three times with 20 mL of 70 % aqueous acetone with sonication for 20 min during each extraction, followed by centrifugation. The supernatants were combined. For FG analyses, the extracts were dried with a rotary evaporator and re-dissolved in methanol. For ET analysis, the extraction was performed as described above, the organic solvent was evaporated and the remaining water phase was freeze-dried. The dried extract was dissolved in 10 mL of water and filtered. Five grams of frozen fresh leaves were milled into a fine powder with liquid nitrogen. An aliquot of 1 g of leaf powder was extracted as described above.

For dry-weight measurement, c.a. 3 g of leaves dried with each processing method and fresh leaves of both cultivars were weighed accurately, dried to a constant weight at 103−105 °C, cooled in a desiccator and weighed.

4.2.4 Extraction and purification of proanthocyanidins (III)

Sea buckthorn purees were accurately weighed, and then extracted three times with solvent consisting of acetone, water and acetic acid (80:19.5:0.5, v/v/v) by sonicating for 15 min for each extraction. The extracts were centrifuged and the supernatants were combined. The acetone was evaporated, then the remaining aqueous extract was defatted with petroleum ether and filtered. The activated Sephadex LH-20 column chromatography was used to purify further the SB puree samples (Yang, Laaksonen, Kallio & Yang, 2016b).

4.3 Chemical analyses

Flavonol glycosides and ellagitannins were analyzed by HPLC-DAD and HPLC-DAD-ESI-MS/MS methods (**I**, **III** and **IV**) according to the method applied previously (Moilanen, Sinkkonen & Salminen, 2013, Yang et al., 2009). The quantitative analyses and preliminary identifications were carried out with reversed phase HPLC-DAD with the aid of reference compounds and UV spectra. The quantification was carried out by using external standards. The corresponding reference compounds were used when commercially available. The most abundant/similar references in the corresponding phenolic compound class were used for those not commercially available. Further identification was conducted by HPLC-DAD-ESI-MS/MS in a mass range of *m/z* 100−1000 (**I** and **IV**) and *m/z* 290–2000 (**IV**) in positive and/or negative ion mode and with the help of literature references of previous research.

The quantitative analysis of PAs was conducted using HPLC-DAD-ESI-MS in negative ion mode (*m/z* 500 to 3000) according to the method applied previously by our group (Kallio, Yang, Liu & Yang, 2014) (**III**). Quantitative analysis of PA oligomers (dimers, trimers and tetramers) was carried out using HILIC-ESI-SIR method and procyanidin B2 as an external standard. The contents of total PAs were determined by BL-DMAC assay as procyanidin B2 equivalent.

Individual sugars and organic acids, as well as EG in the SB juices were determined by gas chromatography (GC) as trimethylsilyl (TMS) derivatives (**II** and **III**). Sorbitol and tartaric acid were used as internal standards. Compounds in SB berries were identified with reference compounds.

4.4 Antioxidant activity measurements of sea buckthorn leaf tea-type infusions (IV)

The hydroxyl radical scavenging activity, the pro-oxidant activity and the ability to chelate iron ions of infusions were measured with the method previously described by Moilanen et al. (2016) with minor modifications. The method is based on the degradation of 2-deoxyribose (2-DR) caused by hydroxyl (HO•) radicals, which are generated in the chain of reactions utilizing Fe^{3+}/ascorbic acid/EDTA/H_2O_2. Some substances can inhibit the degradation of deoxyribose by scavenging HO• (Gutteridge & Halliwell, 1988). The pro-oxidant activities of the tea-type infusions were evaluated based on their ability to reduce Fe^{3+} ions by omitting the addition of ascorbic acid from the assay. Moreover, the metal chelating abilities of these infusions were determined using 2-DR assay, but by omitting EDTA for this purpose. 160 µL of infusions were used instead of pure compounds (Moilanen et al., 2016). For the metal chelation ability measurement, the infusions were diluted (1:9) with water. A Hidex Sense microplate reader (Hidex, Finland) was used to measure the absorbance.

4.5 Sensory evaluation

All the sensory analyses were performed in the sensory laboratory in accordance with the ISO 8589-1988 standard.

The threshold detection of EG in water, and the contribution of EG to sensory profiles of SB model juices/juices were studied with an untrained panel of consumers (**II, Table 4**). The sensory profile of purees were studied with trained panels (**III**, **Table 4**). The general guidelines for the selection, training and monitoring of panels (ISO 8586-1, 1988) were complied. The descriptors were generated following DIS 11035 standards during independent training sessions and the panels were familiarized to the usage of sensory attributes (**Table 5**) and the intensity scale (1−7, **II**; 0−10, **III**).

For the EG (**II**) and SB purees (**III**), at least sweetness, sourness, bitterness and two kinds of astringency were used, and 'Total intensity of flavor' and 'Sharpness' were added in the sensory evaluation of purees. The threshold of EG in water was investigated with Duo-Trio tests, and the contribution of EG, sugars and acids to the taste profile of the constructed model juices were studied with Project Mapping together with Ultra-Flash Profiling (Deegan et al., 2014)(**Table 4**). In study III, the samples were evaluated in randomized orders during three parallel sessions with the help of anchored reference compounds (**Table 5**). The data of threshold and Projective Mapping was carried out on paper. The other data was collected by Compusense-*five* data collection software (version 5.6, *Compusense*, Guelph, Canada).

Prior to chemical analysis (**IV**), four kinds of tea-type beverages, which were processed by the method of FD, S+HT, S+DT and LT, and the infusion of one commercial green tea were prepared and presented to consumers in a preliminary sensory evaluation. The panelists were asked to describe color, aroma and taste of these samples and compared with the green tea.

Table 4. Samples, methods and panelists in the sensory evaluations.

Paper	Sample descriptions	Sensory methods	Methodologies	Number of samples	Number of attributes	Number of assessors/training	Age
II	EG water solutions	Detection threshold measurement (Duo-Trio test)	The panelists were forced to identify one of the two coded samples differing from the reference (water) in taste. Whenever a panelist chose the sample correctly, the same samples were evaluated again to verify the difference. Whenever a panelist selected a wrong sample, the coded samples of EG of the next higher concentration were evaluated.	7	*	29/No	20-52
II	Model SB juices in water	Projective Mapping	The panel was asked to write notes describing the attributes of the samples with their own words, an A3 sized paper was used for placing the samples according to the similarities and differences in sensory profiles.	8	**	44/No	20-52
II	EG water solutions, SB juices, SB juices+ EG	Descriptive ratings on scales	The panelists were asked to taste seven samples and to rate five attributes on a category scale from 1 (not at all) to 7 (very strong). Finally, possible similarities were described between the juice samples and the first sample (EG solution in water). And the most pleasant and unpleasant juice samples were chose.	7	5	45/No	23-52
III	SB purees	Generic Descriptive Analysis	The intensities of the attributes were rated on a continuous graphical scale, from 0 (none) to 10 (very strong) with the help of anchored reference samples (Table 7). The samples were mixed and divided into aliquots of 2 mL in 50 mL transparent plastic beakers covered with lids. The samples were evaluated in triplicate in separate sessions as blind coded and in randomized order.	6	8	12/Yes	23-38
IV	SB leaves tea-type beverages	Description of samples	The panel was asked to describe the color, aroma and flavor of the SB leaves tea-type infusions and the commercial green tea reference. 10 mL of each sample were presented in 50 mL transparent glass beakers in randomized order. The panelists were instructed to take a sip of sample in mouth, to swirl it around in the mouth briefly and to write notes on a blank sheet describing the attributes of the samples with their own words.	5	**	30/No	22-48

* No information provided concerning the sensory quality, however, panelists were asked to describe the quality after completing the threshold test
** Assessors used their own descriptors and attributes

Table 5. Sensory attributes and their descriptions with reference samples.

Paper	Attribute	Verbal Definitions	
II	Sweetness	Resembles the taste found in sugar, candy	
II	Sourness	Lemon, lime	
II	Bitterness	Black coffee, cocoa	
II	Mouth-drying astringency	Drying mouth feeling	
II	Puckering astringency	Shrinking mouth feeling	
		Description	Reference
III	Sweetness	Sweet taste	2.0 % Glucose (VWR, Belgium)
III	Sourness	Sour taste	0.07 % Malic acid (Sigma, St. Louis, Mo)
III	Bitterness	Bitter taste	0.07 % Caffeine (Alfa Aesar GmbH&CoKG, Germany)
III	Puckering astringency	Puckering mouthfeel	0.10 % $AlSO_4$ (Alfa Aesar GmbH&CoKG, Germany)
III	Mouth-drying astringency	Drying mouthfeel	0.20 % Tannic acid (Sigma, St. Louis, Mo)
III	Total intensity of flavor	Perceived first impression of flavor in mouth	–
III	Sharpness	Sharp, acidic, and tangy mouthfeel	High-carbonic acid mineral water (Olvi Vichy, Finland)
III	Aftertaste	Right after swallowing	–

4.6 Statistical analyses

Statistical analyses and multivariate models were performed using SPSS 22.0 (SPSS Inc., Chicago, IL) and Unscrambler X, version 10.3 (CAMO Software, Oslo, Norway), respectively. A one-way analysis of variance (ANOVA) together with suitable post-hoc tests: Tukey's *t*-test or Tamhane test ($p < 0.05$) and independent-sample t test were performed to compare the content and composition of FGs of SB berries/purees in different subspecies, cultivars, growth locations and altitude (**I** and **III**). These analyses were also used to compare sugars and fruit acids at different cultivars and harvest times (**II** and **III**), PAs at different cultivars (**III**), and the ETs of SB leaves "tea" at different cultivars and processing methods (**IV**). Three-way ANOVA was used to analyze sensory results with samples as fixed factors and sessions and panelists as random factors (**III**). Cochran's Q and McNemar's tests were used for the analysis of the frequency data of like/dislike (**II**). Bivariate (Pearson's Correlation Coefficients) and partial correlation analyses were applied to investigate the correlation coefficients among content of FGs in SB berries, latitude and altitude of growth sites (**I**).

Unsupervised classification with PCA models was created to further examine the variation of chemical variables within the two subspecies of SB berries (**I**), and used to investigate variations in the compositional profiles of SB leaves tea-type infusions and residues in the study (**IV**). The PCA models were also applied to analyze the description frequency of SB leaf tea-type infusions from different drying methods and from two cultivars, and used to investigate the contribution of phenolic variables to AAs variables in the tea-type infusions (**IV**). Supervised classification with PLS-DA model was used to explain the difference between ssp. *sinensis* berries and ssp. *mongolica* berries, according to the composition and content of FGs in berries (**I**). PCR was used to analyze the results of the Projective Mapping to determine the interactions between the panelists' sample and frequencies of sensory descriptors (**II**). PLS regression was used to examine the interactions between the compositional variables and the averaged sensory attributes and the frequencies of like and dislike in the SB juices (**II**). It was also applied to investigate relationships between the compositional variables, some ratios between compounds and the averaged sensory attributes in the SB purees (**III**).

5 RESULTS AND DISCUSSION

5.1 Chemical composition

5.1.1 Flavonol glycosides

In total, 26 FGs were identified or tentatively identified in the extracts of SB berries (**I**), and ten major FGs identified were quantified in the extracts of SB purees (**III**). 25 FGs were identified or preliminary identified in the extracts of SB leaves (**IV**) (**Table 6)**. Identification of many phenolic compounds was tentative due to the lack of reference compounds or literature references. The contents of FGs ranged from 23 to 250 mg/100 g FW in berries. The long storage may induce degradation of FGs to the corresponding aglycone. FAs were not found in significant quantities in SB berry samples. This may indicate that the FGs in the berries of SB are generally stable during storage. Yields of the FGs varied between 9.7 mg and 11.7 mg/100 mL in leaf infusions, and the contents ranges were 5.8–7.4 and 13.1–14.1 mg/g DW in the infusion residues and fresh leaves, respectively. The total content of FGs in SB leaves was much higher than reported in the leaves of green tea, oolong tea, and black tea (2.3–5.7 mg/g DW)(Jiang et al., 2015). Glycosides of isorhamnetin represented the highest percentage as well as the majority of compound diversity. Isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside were the two major FGs in all samples analyzed (**I, III** and **IV**). Isorhamnetin and quercetin were the predominant aglycones in SB berries and purees. However, the FGs profiles of leaves were significantly different compared to berries/purees. For example, specific for leaves was the presence of kaempferol derivatives (**IV**). Free FAs were not found in significant quantities in leaf samples analyzed.

5.1.2 Proanthocyanninds and ellagitannins

In SB purees (**III**), no multi-charged molecular ions of PAs were found. Only single-charged molecular ions of PAs with DP from 2 to 4: dimers, trimers and tetramers were detected. The content of PA dimers, trimers, tetramers was only a small proportion (0.30−14.4%) of the total PAs (23.0−47.4 mg/100 g FW), in the range of 0.14–3.50, 0.22–1.97 and 0.12–1.71 mg/100 g FW, respectively. All the compounds represented the B-type PAs.

The content of PA dimers, trimers, tetramers and total PAs appeared to be less in SB purees compared with those in berries (Yang, Laaksonen, Kallio & Yang, 2016b). We speculate that the majority of PAs in SB berries, especially those with DP > 4, are mainly present in the seeds. It has been shown that the subunit composition of PAs is different in the seeds and skin of grapes (Downey, Harvey

& Robinson, 2003). This indicates that different fractions of SB may also have different profiles of PAs.

In paper **IV**, six main ETs e.g. casuarinin, stachyurin, hippophaenin B, hippophaenin C, pedunculagin and casuarictin were detected in SB leaf extracts (**Table 6**). The total amounts of ETs were 9.7–21.7 mg/100 mL in "tea" infusions, 4.0–15.7 mg/g DW in "tea" residues and more than 30 mg/g DW in fresh leaves. The total concentrations of ETs may vary widely between individual SB plants (Suvanto et al., 2018). Casuarinin and stachyurin were the most abundant compounds, whereas hippophaenin B, hippophaenin C and pedunculagin were detected in significantly lower contents in all the samples. The level of total ETs in residues was about a half of that in fresh leaves.

Table 6. Phenolic compounds detected by HPLC-DAD-ESI-MS/MS from the extracts of sea buckthorn berries and leaves.

Com pounds	λ max (nm)	$[M+H]^+$ (*m/z*)	$[M-H]^-$ (*m/z*)	Other ions in MS (*m/z*) [a]	MS/MS[b]	Tentative identification[c]	Compound found in studies
1	254,345	773		<u>303</u>, 465, 627	465, 611, 773	Qu-S-Rh	I, IV
2	256, 355	773		<u>303</u>, 465, 611, 612		Qu-3-S-7-Rh*	I, III, IV
3	257, 346			<u>317</u>, 287, 479, 579		Is Gly I	IV
4	255, 354	787	785	<u>317</u>, 479, 641	317, 479, 641, 787	Is-R-G	I
5	254, 351	787		<u>317</u>, 479	479, 641	Is-S-Rh	IV
6	256, 356	787		<u>317</u>, 463, 464, 625		Is-3-S-7-Rh *	I, III, IV
7	250, 350	449		<u>303</u>, 317	449	Qu-Rh	I, IV
8	255, 351	611		<u>303</u>, 449	449, 611	Qu-He-Rh I	IV
9	252, 350	611		<u>303</u>, 449, 463	449, 611	Qu- He-Rh II	IV
10	256, 357	611	610	<u>303</u>, 466		Qu-3-R*	I, III, IV
11	255, 345	625		<u>317</u>, 463	463, 625	Is-He-Rh I	IV
12	253, 332	611		<u>303</u>, 449	449, 465, 611	Qu-He-Rh III	IV
13	255, 355	465	464	<u>303</u>		Qu-3-G*	I, III
14	254, 351	625		<u>317</u>, 463	463, 625	Is-He-Rh II	IV
15	252, 364	595		<u>287</u>, 433	449,595	Ka-3-He-7-Rh	IV
16	254, 352	625		<u>317</u>, 463		Is-3-G-7-Rh *	I, III, IV
17	253, 344	463		317	463	Is-Rh	I, IV
18	252, 330	611		<u>303</u>, 317 , 463	465, 611	Qu-He-Rh IV	IV
19	254, 337	595		317	463, 595	Is-Pe-Rh	I, IV
20	253, 346	625		287, <u>317</u>, 479	625	Is-R	I, III, IV
21	254, 353	625		<u>317</u>, 479		Is-3-R *	I, III, IV
22	347	449		287	449	Ka-He	IV
23	253, 350	479		317		Is-3-G *	I, III, IV

24	266, 313		287, 303, 313, 595, 617	595	ka-*p*-coumaroylhe I	IV
25	266, 312		287, 303, 595, 618		ka-*p*-coumaroylhe II	IV
26	336		287, 317, 454, 595, 617	595	Ka Gly I	IV
27	253, 353		317, 287, 611, 629, 791	629, 791	Is Gly II	IV
28		783	391, 481, 301		Pedunculagin	IV
29		935	458, 467		stachyurin	IV
30		1103	520, 529		Hippophaenin C	IV
31		935	467		Casuarinin	IV
32		1103	529		Hippophaenin B	IV
33		935	467		Casuarictin	IV

[a] Major fragments in the fragmentation process are underlined.
[b] The MS/MS data were obtained by scanning the daughter ion of [M+H]+.
[c] Compounds with * in the column were identified with reference compounds; the others were identified based on UV and mass spectra.

5.1.3 Sugars, ethyl *β*-D-glucopyranoside and fruit acids

Fructose and glucose were the two major sugars, and malic and quinic acids were the major fruit acids in the SB berries/purees studied (**II** and **III**). A significant increasing trend was found in the contents of EG in ‘Terhi’ and ‘Tytti’ during August-October ($p < 0.05$, **II**). Results of the present research revealed clear and significant differences in the contents of sugars, EG and acids in six cultivars and different harvest times (**II** and **III**, respectively).

5.2 Influence of genotype, growth conditions and processing

5.2.1 Comparison of subspecies and cultivars

In paper **I**, the total content of FGs, as well as the contents of all individual major compounds, were significantly higher in ssp. *sinensis* than in ssp. *mongolica.* Among the cultivars of ssp. *mongolica*, the berries of ‘Oranzhevaya’ had the lowest content of total FGs (23 mg/100 g FW), whereas the berries of ‘Prevoshodnaya’ were the richest in total FGs (80 mg/100 g FW).

In paper **III**, ‘Leikora’ contained higher contents of isorhamnetin-glucoside-rhamnoside, isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside, isorhamnetin-3-*O*-rutinoside, and total FGs compared with other cultivars ($p < 0.05$). In contrast, ‘Avgustinka’ had the lowest content of these compounds ($p < 0.05$). The content of total FGs in ‘Leikora’ was nearly 3-fold of the level in ‘Avgustinka’. ‘Terhi’ contained the highest ratio of Is/Qu, whereas 'Trofimovskaya' the lowest ($p < 0.05$).

In paper **IV**, there were significant differences in the contents of individual ETs in the infusion samples between the two cultivars, except for stachyurin ($p < 0.05$). Casuarinin was detected in significantly lower quantities in 'Tytti' than in 'Terhi'. The total content of FGs and the contents of some individual FGs were significantly higher in the residue samples of 'Terhi' than in those of 'Tytti' ($p < 0.05$). The ratio between isorhamnetin and quercetin glycosides in 'Terhi' was 2.3 and 1.9 in 'Tytti', and the same trend was detected in the infusions and in the fresh leaves.

The berries and purees of SB had similar profiles of FGs (Ma et al., 2016, Ma et al., 2017), but differed in the leaf samples due to the presence of kaempferol derivatives. Results of this study revealed clear differences in the content and composition of FGs between the subspecies/cultivars. The profile of FGs in SB may be a useful chemotaxonomic feature distinguishing SB of different origins (Chen, Zhang, Xiao, Yong & Bai, 2007).

5.2.2 Effect of growth conditions on flavonol glycosides (I)

The FGs contents in the berries from Kittilä (North Finland) were significantly higher than the contents in the corresponding cultivars ('Avgustinka', 'Botanicheskaya', 'Trofimovskaya' and 'Pertsik') cultivated in Turku (south Finland) (77 *vs* 49 mg/100 g FW, respectively). Among all weather variables studied from south to north, the sum of the daily mean temperatures (from the start of growth season to harvest) was the most important variable, which correlated negatively with the accumulation of FGs in berries (Zheng et al., 2016). The total FGs content of 'Prozcharachnaya' was significantly higher in samples from Kittilä than from Québec. Interestingly, FGs with simpler structures, especially isorhamnetin-3-*O*-glucoside, were rich in Québec and Turku, whereas the glycosides with complex sugar moieties were more abundant in Kittilä. Among the berries of the 6 growth sites of ssp. *sinensis*, the berries from Sichuan were distinguishable with higher content of FGs than berries from other locations.

A positive correlation has been reported between latitude and the content of phenolic compounds in Finnish berries (Latti et al., 2009, Lätti et al., 2007). Similar trends were observed in most of the berries of ssp. *mongolica*. However, in the berries of ssp. *sinensis*, a negative association was found between latitude and the content of most FGs and the total contents of FGs. This indicates the importance of interaction between genetic background and environmental factors. The contents of almost all flavonol compounds correlated negatively with latitude but positively with altitude ($p < 0.01$). Altitude influences the quality of radiation, which has been shown to affect the composition and the concentration of UV-absorbing compounds in plants (Cockell, north & Herrera, 2000, Davik, Bakken, Holte & Blomhoff, 2006, Kolb et al., 2003). Despite some outliers, increasing trends were seen in the contents of FGs as the altitude increased and as the latitude decreased in the Chinese berries (**Figure 7**).

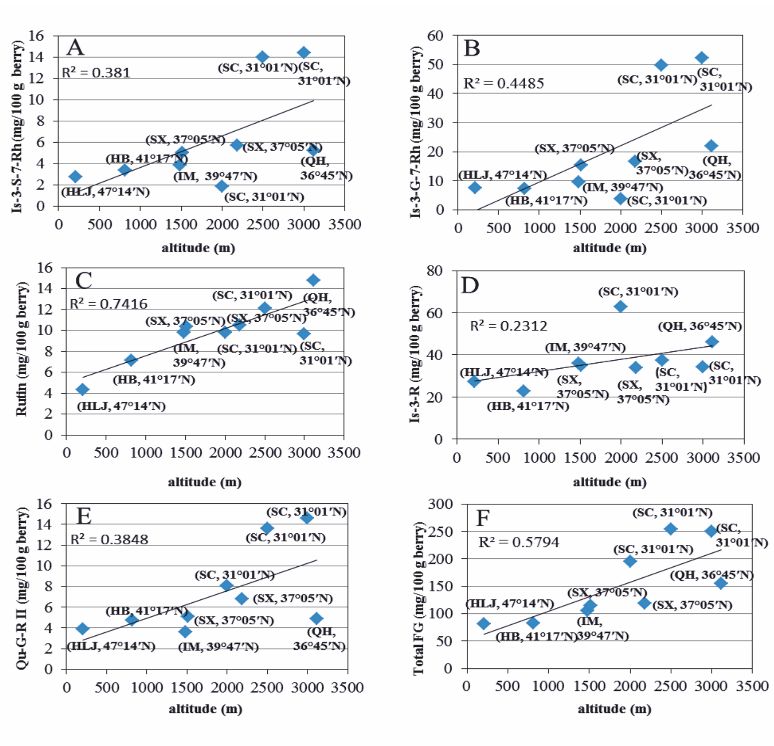


Figure 7. Correlations between altitude and contents of isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside (A, Is-3-S-7-Rh), isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside (B, Is-3-G-7-Rh), quercetin-3-*O*-rutinoside (C, Rutin), isorhamnetin-3-*O*-rutinoside (D, Is-3-R), quercetin-glucoside-rhamnoside II (E, Qu-G-Rh II) and total flavonol glycosides (F, Total FG). Growth sites and latitudes of wild Chinese berries were indicated in parentheses on the plot. Reprinted from original publication **I**, with permission from Elsevier.

5.2.3 Comparison of drying methods (IV)

Generally, the contents of ETs varied significantly among the different drying methods studied, whereas less effect was seen on the contents of FGs. The content of hippophaenin B varied among different drying methods by close to 4-fold in the infusions of ‘Tytti’. The total concentration of ETs ranged between 9.7 and 21.7 mg/100 mL, and the processing of S+HT resulted in the highest content of total ETs and total phenolics in the infusions. Air-drying led to the highest content of hippophaenin B, hippophaenin C and casuarictin in the infusions. Only few individual FGs showed significantly varying contents among different drying methods, whereas there were no significant differences in the contents of total FGs. This may indicate that the FGs of SB leaves are generally stable during different drying process. This is consistent with previous reports that FGs are more resistant to heat processing than some other groups of phenolics, such as anthocyanins (Mäkilä, Laaksonen, Kallio & Yang, 2017).

5.3 Sensory properties

5.3.1 Taste threshold of ethyl *β*-D-glucopyranoside in water (II)

The threshold of EG in water was determined resulting in a threshold 1.1 ± 1.3 g/L (0.005 mol/L), calculated as a mean of BET (Best estimate of individual threshold). When compared to the reference (water), 76% of panelists reported that the main difference was bitterness. The observed threshold of EG was lower than the levels at which this compound is naturally present in SB juices of *H. rhamnoides* ssp. *rhamnoides* (Yang, 2009). Hence, the naturally present EG may be a contributor to the flavor of the berries of SB, at least in this subspecies.

5.3.2 Sensory profiles of sea buckthorn purees (III)

Overall, all the puree samples of six SB cultivars were perceived as notably sour, bitter and puckering astringent, as well as sharp. 'Trofimovskaya' puree was described as the least sour, puckering astringent and sharp among the cultivars, whereas 'Hergo' was perceived as opposite in these attributes. Similarly, the purees of 'Terhi', 'Tytti' and 'Leikora', had higher intensities of sourness, astringency and total flavor, whereas that of 'Avgustinka' contained lower scores of sourness and sharpness ($p < 0.05$). Generally, no significant differences were detected in sweetness and bitterness among all cultivars. Whereas the cultivars 'Trofimovskaya' and 'Avgustinka' were sweeter and less sour and astringent, significantly different from the other cultivars based on the scores of sensory attributes.

5.3.3 Sensory evaluation of tea-type infusions (IV)

The panelists described mainly the color of the infusions as brown/dark yellow, the aroma as stronger berry, fishy and fermented, and the flavor as sweet, mild and fishy, while lacking the astringent and bitter flavor of green tea. Use these descriptors and the lack of negative descriptors indicated that tea-type infusions prepared from the SB leaves might be acceptable to consumers.

5.4 Antioxidant activities of tea-type leaf infusions (IV)

In general, the tea-type infusions in this study presented intense antioxidant activities, and 'Terhi' samples showed higher activities than 'Tytti'. (**Figure 8**). The radical scavenging activities of two non-thermal drying methods (air- and freeze-drying) are similar and stable (**Figure 8A**). The thermal drying methods (LT, S+HT and S+DT) present higher pro-oxidant and lower radical scavenging activities than the non-thermal ones in the infusions. It seems that harsh drying methods increase the pro-oxidative effects of tea-type infusions, and the AAs show sensitivity to drying temperatures in tea infusions. Moreover, the leaves were treated with steam and thermal processing (S+HT or S+DT in **Figure 8C**) led to the highest pro-oxidative activities and higher deviations in the infusions of both cultivars. These changes were suggested to be due to modifications in the total phenolic content and profile by phenolic oxidation or polymerization caused by thermal processing (Randhir, Kwon, & Shetty, 2008). The deviations might be caused by some degradation products produced during heat treatment.

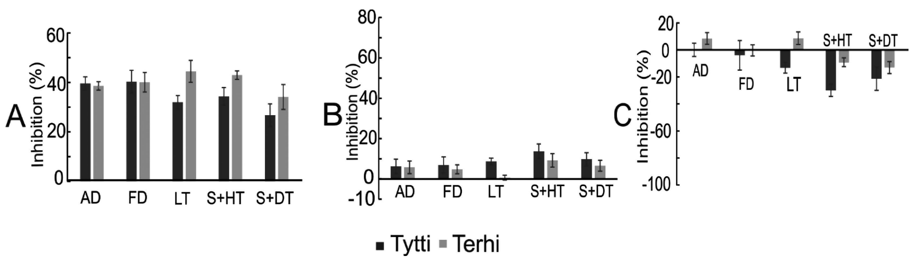


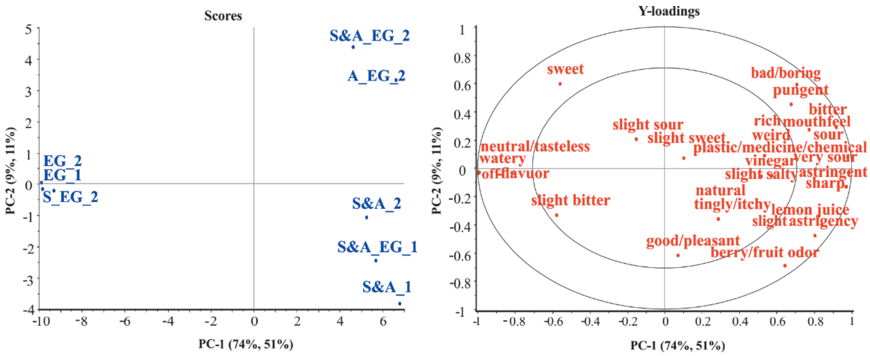
Figure 8. Radical scavenging activities (A), metal chelating abilities (B) and pro-oxidant activities (C) of sea buckthorn leaf tea-type infusions. Negative inhibition values in C indicate pro-oxidant activity. Adapted from the original publication **IV**.

5.5 Combining the data

5.5.1 Compounds contributing to sensory properties

Principal component regression (PCR) was used to analyse the sensory contribution of EG in the eight aqueous mixtures of acids, sugars and/or EG at different concentrations (**Figure 9**, **II**). PLS regression models were used to combine chemical variables or ratios of chemical variables and sensory data in papers **II−III** as well as to investigate correlations between the chemical variables and AAs in paper **IV**. Only the first two factors of the model are shown in the figures.

In PCR models (**Figure 9**), all the samples with acids correlated with 'very sour', 'bitter', 'astringent' and 'pungent' variables in the loading plot. In contrast, the model samples without any acids were described with attributes 'off-flavour', 'watery', 'sweet' and 'slightly bitter'. Only sample S&A_EG_2 was described to be somewhat more bitter than sample S&A_2 in the second principal component. It might be due to the presence of EG in the sample of S&A_EG_2. The contribution of EG to bitterness was not sufficiently significant according to results of evaluation by the panel. Due to the high concentration of organic acids, the potential bitterness of EG may be suppressed by the higher intensity of sourness in the overall profile (Keast & Breslin, 2003).



Abbreviation	Sensory sample	Malic acid	Quinic acid	Fructose	Glucose	EG	Model
S&A_1	sugar&acid water solution (No EG)	9.6	3.9	0.3	2.4	0	
S&A_EG_1	sugar&acid water solution + EG	9.6	3.9	0.3	2.4	0.4	1
EG_1	EG water solution	0	0	0	0	0.4	
S&A_2	sugar&acid water solution (No EG)	9.5	2.6	0.4	1.7	0	
S&A_EG_2	sugar&acid water solution + EG	9.5	2.6	0.4	1.7	1.0	
EG_2	EG water solution	0	0	0	0	1.0	2
A_EG_2	acid water solution + EG	9.5	2.6	0	0	1.0	
S_EG_2	sugar water solution + EG	0	0	0.4	1.7	1.0	

Figure 9. Principal component regression scores and Y-loadings plots of coordinates (X, not shown in the figure), descriptors of samples (Y, red font) in the eight sugar-acid mixtures (blue font). Reprinted from the original publication **II**, with permission from Elsevier.

In order to investigate the role of EG in SB juices, sweetness, sourness, bitterness and the two astringent properties (mouth drying and puckering) were rated in the juices with and without added EG (**Figure 10, II**). The only statistically significant sensory difference was found in the sourness between juices without (TeAu15) and with an addition of EG (TeAu15 + EG 2) ($p = 0.01$). It is possible that the bitterness of the added EG suppressed the high intensity of the sourness (Keast & Breslin, 2003). A significant increase in bitterness was perceived in the juice of 'Tytti' after the concentration of EG was increased. This indicated that EG contributed to the bitter taste of the juice. Significant differences were found in the sensory profiles between the juices before and after the addition of EG. This might be due to the different profiles of sugars and acids in the juice of 'Terhi' and 'Tytti'. Moreover, it is important to note that other compounds such as phenolic compounds may contribute to the sensory properties of these juices perceived astringency and bitterness (Hufnagel & Hofmann, 2008a), although they were not detected in this study. After rating the attributes, bitterness was perceived mainly in the aqueous EG solution by most of the panelists.

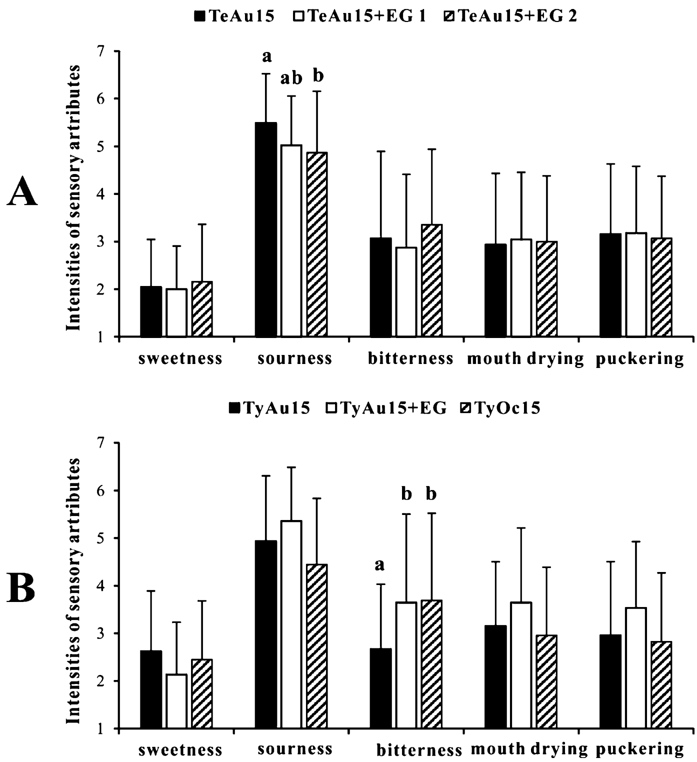


Figure 10. Sensory attributes and their intensities (scale 1–7) in the juices with and without added EG by the sensory panel (n=45). (A) TeAu15 ('Terhi' collected in August, 2015; EG content 0.6g/L), + EG1 (1.7 g/L) and + EG2 (4.5 g/L); (B) TyAu15 ('Tytti' collected in August, 2015; 1.6 g/L), + EG (3.3 g/L) and TyOc15 ('Tytti' collected in Octorber, 2015; 19.8 g/L). Statistical differences between the samples are marked with different letter (a–b) ($p < 0.05$). Reprinted from the original publication **II**, with permission from Elsevier.

As shown in PLS regression model in **Figure 11**, glucose and fructose were the major sugars contributing to the sweetness in SB juices/purees (**II** and **III**). L-quebrachitol showed only little correlation with any of the sensory attributes.

The main contribution of EG was the bitter taste, which was closely correlated to the ratios of EG/acid and EG/sugar (**II**). Malic acid was the major organic acid contributing to the sourness (**II** and **III**). Due to the intense sourness in the SB juices/purees, the ratio of sugars/acids is often considered as a better predictor of sourness and/or sweetness of berries and berry products (Tang, Kälviäinen & Tuorila, 2001, Tiitinen, Hakala & Kallio, 2005). Malic acid and isorhamnetin glycosides, especially isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside, were closely related to the astringent attributes in the different purees, whereas PAs or quercetin glycosides had less impact (**III**). Moreover, comparing individual variables alone, the ratios between contents of acids and phenolic compounds were more important predictors of bitterness (**III**).

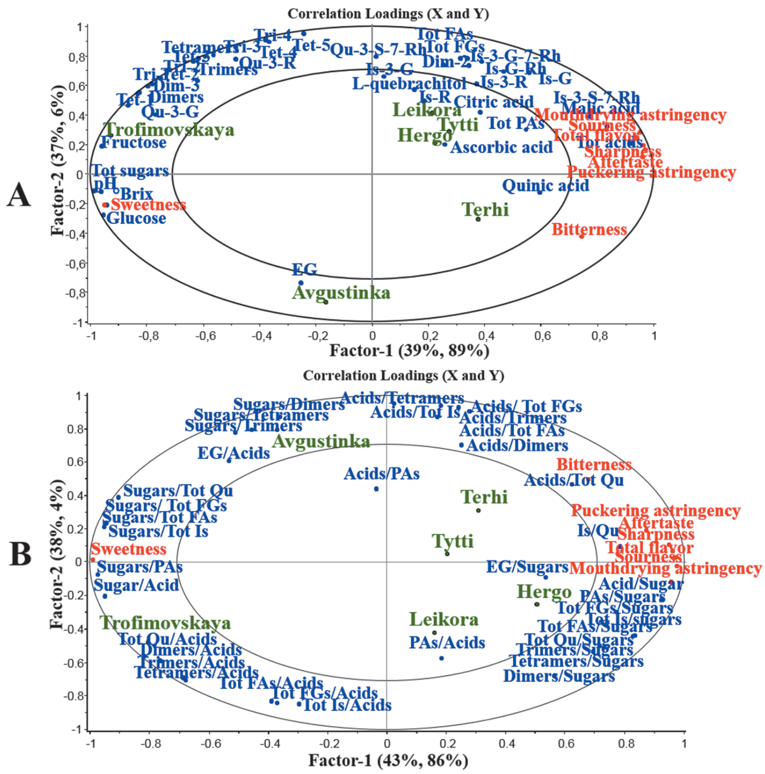


Figure 11. Partial least squares (PLS) regression model showing the interactions between chemical variables as X-variables (blue font) or ratios of chemical variables as X-variables (blue font) and sensory profiles as Y-variables (red font) in six puree samples (green font). Reprinted from the original publication **III**, with permission from American Chemical Society.

5.5.2 Sensory-chemical factors contributing to liking and disliking of juices (II)

The most-liked juice was TyAu15 within the 6 juice samples, while TeAu15, TeAu15 + EG 2 and TyAu15 + EG were selected as most-disliked juice samples. As shown in PLS regression model in **Figure 12,** the liking of SB juices was related positively to citric acid but correlated negatively with EG as well as the ratios of EG/sugars and EG/acids. The contents of malic acid, quinic acid, ascorbic acid and the total acids were found to correlate positively with sourness and puckering astringency, but they correlated only weakly with liking. To some extent, the concentration of EG was found to correlate positively with the intensity of the bitterness perceived in the juices and disliking of juice to consumers. However, sometimes consumers in beverages enjoyed complex and balanced tastes, and bitterness may help to balance the flavor profile and add complexity and enjoyment of fruit juices (Geertsen et al., 2016).

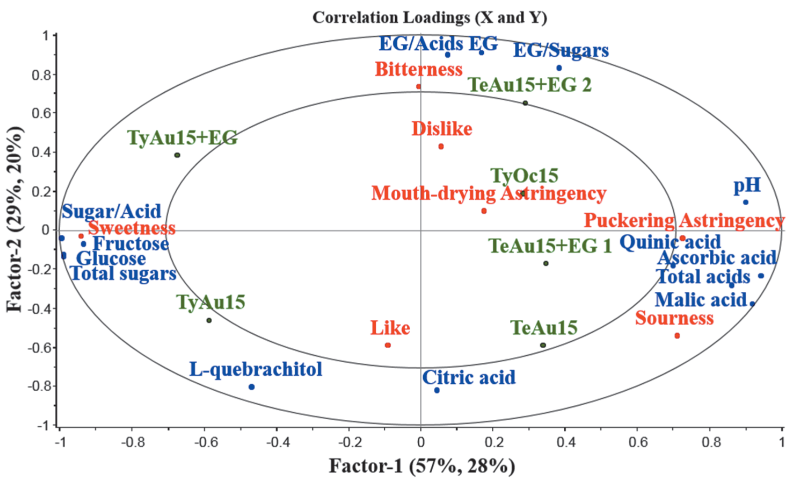


Figure 12. Correlation loadings plot of the partial least squares (PLS) regression model showing the interactions between chemical variables (X-variables, n=14; blue font) and sensory profiles (Y-variables, n=7; red font) in six juice samples (green font). The sample abbreviations refer to **Figure 10**. Reprinted from the original publication **II**, with permission from Elsevier.

5.5.3 Correlation between phenolic compounds and antioxidant activities (IV)

Most ellagitannins, such as casuarictin, hippophaenin B, stachyurin, total ETs, and total phenolics correlated strongly positively to the metal chelation abilities and negatively to the pro-oxidant activities. Is-R and Is-3-G correlated positively with pro-oxidant activities. Some quercetin glycosides such as Qu-He-Rh III and Qu-He-Rh IV were closely associated with radical scavenging activities, whereas kaempferol glycosides and part of quercetin glycosides had a weak correlation with AAs. Generally, the results suggest that the AAs of ETs are more likely due to their capacity for chelating metal ions, and FGs mainly influence on radical scavenging activities. Some previous publications also reported strong correlations between phenolics and the AAs of plant extracts evaluated by different assays (Liaudanskas et al., 2014, Luximon-Ramma et al., 2002). However, the AAs of the extracts was affected by many factors, and no clear association was found between the AAs of extracts and the relative proportion of FGs and ETs despite the fact that these compounds are generally known to be potent antioxidants.

6 SUMMARY AND CONCLUSION

Isorhamnetin was the most abundant aglycone and represented a majority of compound diversities of flavonol glycosides in SB berries and leaves. The content and profile of FGs in SB berries were highly dependent on the subspecies and cultivars. Moreover, they were significantly influenced by the altitude and/or latitude of the growth sites. Besides FGs, ellagitannins were also investigated in SB leaves and leaves products. The contents of ETs varied significantly among the different processing methods, whereas less effect was seen on the contents of FGs. The knowledge on specific FGs and ETs in SB berries and leaves can provide an accurate dietary intake of bioactive compounds in various clinical studies and a better estimation of potential health benefits of sea buckthorn.

The results showed that SB of different genetic backgrounds and harvest times had significantly different sensory profiles due to different chemical compositions. EG is a bitter compound, which positively correlated with the disliking of SB juices. Malic acid and isorhamnetin glycosides were the major compounds responsible for the astringency in SB purees. Considering the content and contribution of EG, the berries of ssp. *rhamnoides* are predicted to be bitterer than those of ssp. *sinensis* and ssp. *mongolica*. SB berries of ssp. *sinensis* may be more astringent than those of ssp. *mongolica*, due to the higher content of isorhamnetin glycosides. Drying process may have less influence on the taste properties of tea-type leaf infusions, as the content of astringent FGs may be stable during the processing.

The conclusions on sensory properties of SB in this study were made based on only a few cultivars, and only some of the taste-active compounds were included. Although volatiles, lipids, polysaccharides and various other compounds will influence the flavor of SB berries and berry products, we only focused on the role of non-volatile bioactive compounds in the research of this thesis.

Sea buckthorn has gained increasing interest as a health food due to the wide range of health benefits. The consumption of SB berries is limited usually because of the special sensory profile. Our findings bring new insights into the sensory profiles of SB berries, especially astringency and bitterness, as well as the major compounds associated with these taste contributes of sea buckthorn. By understanding the relationship of chemical factors and these sensory attributes, it is possible to improve the sensory properties and consumer acceptance of SB products, which is crucial for increasing the utilization of SB in the food industry and providing guidance for SB breeding and cultivation.

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