DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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The word technology comes from Greek:
techne "science of craft"
logial "art, skill, cunning of hand"

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ABSTRACT

In the food industry, berries are conventionally processed into juices, jams, jellies, purées, concentrates and extracts. Phenolic compounds play an important role in the health effects and sensory properties of berry products. The factors influencing the stability of phenolic compounds are the type of process and process parameters, the food matrix (pH, presence of oxygen, enzymes, pectins, sugars and metals), and storage conditions. Anthocyanins are especially vulnerable during processing and storage, resulting in pigment degradation and formation of anthocyanin-tannin/flavan-3-ol polymers. Flavonol glycosides, ellagitannins and derivatives of phenolic acids are hydrolyzed into free aglycones and acids, and the degree of polymerization (DP) of proanthocyanidins is lowered. The decomposition of phenolic compounds, especially anthocyanins, may compromise the sensory and nutritional food quality.

Processing berries into juices results in 20–30% of press residue, producing significant ecological problems when discarded as waste. The press residue contains high amounts of phenolic compounds and could be a valuable source of bioactive ingredients. Thermal and non-thermal techniques exploiting cavitation (hydrothermodynamic processing), microwave heating, electroporation (pulsed electric field processing), and high pressure have the potential to provide berry products with improved yields of phenolic compounds and less decomposition due to lower treatment temperatures and shorter exposure to heat during the treatment. These processes are viable alternatives during berry blanching and juice pasteurization, and also for extraction of phenolic fractions from the press residue.

In the practical work of the thesis, the impact of various juice pressing technologies and storage conditions were studied on the chemical composition and sensory properties of black currant (*Ribes nigrum* L.) juices. During juice processing, berries were pressed with and without the application of supplementary enzymes. A two-phase juice extraction process yielded “Non-Enzymatic Berry”- and “Enzymatic Press Residue”-juices, which were compared with the conventional enzyme-assisted juice. The high phenolic content resulted in an increased astringency and bitterness in the enzymatically pressed juices. Native undisrupted pectins in the “Non-Enzymatic Berry”-juice reduced the astringency. The lowest content of phenolic compounds in the “Non-Enzymatic Berry-juice” led to the lowest stability of the phenolic composition during pasteurization and storage. The most notable decrease was observed in the monomeric anthocyanins, and the most significant increase in

phenolic acids. Generally, the stability of anthocyanins is mostly influenced by the storage temperature, whereas other phenolic compounds are sensitive to both temperature and light. Light induced the conversion of (*E*)-*p*-coumaric acid derivatives into the corresponding (*Z*)-isomers. Flavonol glycosides were more stable, compared to anthocyanins and hydroxycinnamic acid derivatives. The chemical and sensory quality of the juices remained rather constant during cold (+4 °C) storage.

Analyses were conducted by high-performance liquid chromatography – diode-array detection – electrospray ionization – mass spectrometry or tandem mass spectrometry (HPLC–DAD–ESI–MS(–MS^2)), high-performance liquid chromatography – diode-array detection – electrospray ionization – quadrupole time-of-flight mass spectrometry (HPLC–DAD–ESI–Q-TOF-MS) and analysis by nuclear magnetic resonance (NMR), after isolation with selective high-performance liquid chromatography. Analyses revealed derivatives of hydroxycinnamic acids: 2-(*Z*)-*p*-coumaroyloxymethylene-4-β-D-glucopyrano-syloxy-2-(*Z*)-butenenitrile, 2-(*E*)-caffeoyloxymethylene-4-β-D-glucopyrano-syloxy-2-(*Z*)-butenenitrile, (*Z*)-*p*-coumaric acid 4-*O*-β-D-glucopyranoside and 1-*O*-(*Z*)-*p*-coumaroyl-β-D-glucopyranose, and also free (*Z*)-*p*-coumaric acid.

New product concepts for snacks were developed using the press residue obtained from different juice pressing processes. The research conducted for this thesis provided important knowledge on the stability of berry phenolics during various conditions of food processing and storage, and introduced sustainable process technologies for utilization of the berry and the side-stream. The results can promote the development of new berry products with enhanced quality.

SUOMENKIELINEN ABSTRAKTI

Elintarviketeollisuus prosessoi marjat mehuiksi, hilloiksi, hyytelöiksi, soseiksi, konsentraateiksi ja uutteiksi. Marjatuotteiden sisältämät fenoliset yhdisteet ovat tärkeitä niiden terveydellisten vaikutusten ja aistittavan laadun kannalta. Fenolisten yhdisteiden stabiilisuus riippuu suurelta osin valitusta tuotantoteknologiasta ja prosessiparametreista, elintarvikematriisista (pH, hapen läsnäolo, entsyymit, pektiinit, sokerit ja metallit) sekä säilytysolosuhteista. Antosyaniinit ovat erityisen herkkiä marjatuotteiden prosessoinnin ja säilytyksen aikana, mikä voi johtaa pigmentin hajoamiseen ja antosyaniini-tanniini/flavan-3-olien polymeerien muodostumiseen. Flavonoliglykosidit, ellagitanniinit ja fenolisten happojen johdannaiset saattavat hydrolysoitua vapaiksi aglykoneiksi ja hapoiksi prosessoinnin ja säilytyksen aikana, ja proantosyanidiinien polymeroitumisasteen on huomattu laskevan. Fenolisten yhdisteiden, etenkin antosyaniinien hajoaminen saattaa heikentää marjapohjaisten elintarvikkeiden aistittavaa ja ravitsemuksellista laatua.

Marjojen prosessointi mehuksi tuottaa noin 20–30% puristejäännöstä. Hyödyntämättömänä puristejäännös aiheuttaa merkittäviä ympäristöongelmia. Jäännös sisältää runsaasti fenolisia yhdisteitä, ja se voisi olla hyödyllinen bioaktiivisten ainesosien lähde. Kavitaatioon (hydrotermodynaaminen prosessi), mikroaaltolämmitykseen, elektroporaatioon (pulssitettu sähkökenttä), ja korkeaan paineeseen perustuvat prosessit mahdollistavat fenolisten yhdisteiden korkeamman saannon ja paremman säilymisen marjatuotteissa hyödyntämällä matalampia lämpötiloja sekä lyhyempiä kuumennusaikoja käsittelyn aikana. Nämä prosessit ovat hyviä vaihtoehtoja marjojen ryöppäykseen ja mehujen pastörointiin, kuten myös fenolisten fraktioiden uuttamiseen puristekakusta.

Väitöskirjan kokeellisessa osassa tutkittiin miten erilaiset mehunpuristusteknologiat ja säilytys vaikuttavat mustaherukkamehujen (*Ribes nigrum* L.) kemialliseen ja aistittavaan laatuun. Mehun valmistuksessa marjoja puristettiin sekä lisäentsyymeillä että ilman entsyymejä. Kaksivaiheinen marjan puristusprosessi tuotti ”ei-entsyymi-mehua” sekä ”entsyymipuristettua puristekakkumehua”, joita verrattiin perinteiseen entsyymipuristettuun mehuun. Entsyymipuristettujen mehujen korkea fenolipitoisuus lisäsi mehun astringoivuutta ja karvautta. Ei-entsyymipuristetun mehun luontaiset pektiinit alensivat mehun astringoivuutta. Ei-entsyymipuristetun mehun alhaisin fenolipitoisuus johti epävakaiseen fenolikoostumukseen pastöroinnin ja säilytyksen aikana. Monomeeristen antosyaniinien pitoisuus laski huomattavasti ja fenolisten happojen pitoisuus kasvoi merkitsevästi.

Säilytyslämpötila vaikutti antosyaniinien stabiilisuuteen huomattavasti, kun taas muut fenoliset yhdisteet olivat herkkiä sekä korkealle säilytyslämpötilalle että valolle. Valo sai aikaan (*E*)-*p*-kumaarihappojohdannaisten muuntumista vastaaviksi (*Z*)-isomeereiksi. Flavonoliglykosidit olivat pysyvämpiä kuin antosyaniinit tai hydroksikanelihappojen johdannaiset. Mehujen kemiallinen ja aistinvarainen laatu säilyi melko vakaana kylmäsäilytyksessä (+4 °C).

Uusia hydroksikanelihappojen johdannaisia tunnistettiin hyödyntäen korkean erotuskyvyn nestekromatografi – diodirividetektio – elektronisuihkuionisaatio – massaspektrometriaa tai tandemmassaspektrometriaa (HPLC–DAD–ESI–MS(–MS^2)), korkean erotuskyvyn nestekromatografi – diodirividetektio – elektronisuihkuionisaatio – kvadrupolilentoaikamassaspetrometriaa (HPLC–DAD–ESI–Q-TOF-MS) sekä ydinmagneettista resonanssianalyysia (NMR), jota ennen yhdisteet oli eristetty valikoivalla korkean erotuskyvyn nestekromatografialaitteistolla. Uudet tunnistetut yhdisteet olivat 2-(*Z*)-*p*-kumaroyylioksimetyleeni-4-β-D-glukopyranosyylioksi-2-(*Z*)-buteeninitriili, 2-(*E*)-kaffeoyylioksimetyleeni-4-β-D-glukopyranosyylioksi-2-(*Z*)-buteeninitriili, (*Z*)-*p*-kumaarihappo 4-*O*-β-D-glukopyranosidi ja 1-*O*-(*Z*)-*p*-kumaroyyli-β-D-glukopyranoosi, sekä vapaa (*Z*)-*p*-kumaarihappo.

Uusia välipalatuotekonsepteja kehitettiin hyödyntäen marjojen puristuksesta ylijäävää puristekakkua. Väitöskirjatutkimus toi uutta tietoa marjojen fenolisten yhdisteiden stabiilisuudesta elintarvikeprosessien ja säilytyksen aikana eri olosuhteissa. Tutkimuksessa esiteltiin myös ympäristöä säästäviä prosessiteknologioita marjoille ja sivuvirroille. Tuloksia voidaan hyödyntää kehitettäessä uusia korkealaatuisia marjatuotteita.

LIST OF ABBREVIATIONS

*a**	(+) redness, (–) greenness
ADF	Acid detergent fiber
ANOVA	Analysis of variance
*b**	(+) yellowness, (–) blueness
DAD	Diode array detector
DP	Degree of polymerization
E	*trans*
EB	Enzymatic Berry
EC	Enzyme Comission
EPR	Enzymatic Press Residue
ESI–MS	Electrospray ionisation – mass spectrometry
HCA	Hydroxycinnamic acid
HHP	High hydrostatic pressure
HPLC	High-performance liquid chromatography
HTD	Hydrothermodynamic
*L**	Lightness; 0 = black, 100 = white
LC–MS	Liquid chromatography – mass spectrometry
NDF	Neutral detergent fiber
NEB	Non-Enzymatic Berry
NMR	Nuclear magnetic resonance
p	*para*
PCA	Principal component analysis
PLS	Partial least squares
PEF	Pulsed electric field
POD	Peroxidase
PPO	Polyphenol oxidase
Q-TOF	Quadrupole-time-of-flight
RT	Room temperature
SEI	Sectional expansion index
UV	Ultra violet
vis	Visible
WAI	Water absorption index
WSI	Water solubility index
Z	*cis*

LIST OF ORIGINAL PUBLICATIONS

I. Laaksonen, O.; Mäkilä, L.; Tahvonen, R.; Kallio, H.;Yang, B. Sensory quality and compositional characteristics of blackcurrant juices produced by different processes. *Food Chem.* **2013**, *138*, 2421–2429.

II. Mäkilä, L.; Laaksonen, O.; Ramos Diaz, J.M.; Vahvaselkä, M.; Myllymäki, O.; Lehtomäki, I.; Laakso, S.; Jahreis, G.; Jouppila, K.; Larmo, P.; Yang, B.; Kallio, H. Exploiting blackcurrant juice press residue in extruded snacks. *LWT–Food Sci. Technol.* **2014**, *57*, 618–627.

III. Mäkilä, L.; Laaksonen, O.; Alanne, A-L.; Kortesniemi, M.; Kallio, H.; Yang, B. Stability of hydroxycinnamic acid derivatives, flavonol glycosides and anthocyanins in black currant juice. *J. Agric. Food Chem*. **2016**, *64*, 4584–4598.

IV. Mäkilä, L.; Laaksonen, O.; Kallio, H.; Yang, B. Effect of processing technologies and storage conditions on stability of black currant juices with special focus on phenolic compounds and sensory properties. *Food Chem.* **2017**, *221*, 422–430.

1 INTRODUCTION

Berries are rich in valuable nutrients such as phenolic compounds, fibers, vitamins and unsaturated fats and oils (Bąkowska-Barczak & Kolodziejczyk 2011; O'Shea et al. 2012). Berries have unique profiles of phenolic compounds (Häkkinen et al. 1999; Määttä-Riihinen et al. 2004), sugars and acids (Mikulic-Petkovsek et al. 2012), as well as of flavor (Laaksonen et al. 2010; Laaksonen et al. 2016) and aroma (Latrasse 1991). Phenolic compounds in berry-based products contribute significantly to the sensory quality, such as bitterness and astringencies and color (Hufnagel & Hofmann 2008; Sandell et al. 2009; Scharbert et al. 2004; Schwarz & Hofmann 2007). Phenolic compounds may also contribute to human health due to strong antioxidative, anticarcinogenic, antimutagenic and anti-inflammatory activities (Mazzoni et al. 2016; Moyer et al. 2002; Zafra-Stone et al. 2007). The stability of phenolic compounds is affected by several factors including high temperature, pH, light, presence of oxygen, enzymes, pectins, sugars and metals (Castañeda-Ovando et al. 2009; Patras et al. 2010; Hartmann et al. 2008; Kalt et al. 2000; Chisari et al. 2007; López-Serrano & Ros Barceló 2002; Meschter 1953; Nikkhah et al. 2007; Brouillard 1982; Castañeda-Ovando et al. 2009).

In the food industry, most of the berries are processed into juices, jams, jellies, purées, concentrates and extracts. Conventional processing of berries involves application of high temperatures to inactivate microbes and oxidizing native enzymes. The juice industry applies supplementary enzymes to enhance juice extraction. Often long storage is applied to the final berry products. Processing and long storage jeopardizes the bioactive compounds of berries, especially anthocyanins (Aaby et al. 2007; Brownmiller et al. 2008; García-Viguera et al. 1999; Hager et al. 2008; Hellström et al. 2013; Holzwarth et al. 2013; Howard et al. 2010; Oszmiański & Wojdyło 2009; Patras et al. 2011; Teleszko et al. 2016; Wicklund et al. 2005; Wilkes et al. 2014)

Consumers have an increasing appreciation of food, which is nutrient dense, fresh, natural, additive free and minimally and sustainably processed (Oey et al. 2008b; Tadapaneni et al. 2014). It is believed that the consumption of berries will increase as a result of new products being brought onto the markets, and of an awareness of a healthy diet (Commission of the European Communities 2006). Processes exploiting electroporation, cavitation, microwave heating and pressure have the potential to better retain the anthocyanins and other phenolic compounds in the berry products, compared to conventional processing, due to the reduced use of heat (Bobinaitė et al. 2015; Chen & Martynenko 2016a; Chen & Martynenko 2016b; Elik et al. 2016; Marszałek et al. 2016; Medina-Meza & Barbosa-Cánovas 2015; Medina-Meza

et al. 2016; Pala & Toklucu 2013; Tadapaneni et al. 2014; Vilkhu et al. 2008). These techniques increase disintegration of the cell membrane, resulting in inactivation of microbes and oxidizing native enzymes. At the same time, the cell disintegration enhances the extraction of phenolic compounds from the berry tissues into the berry products. These technologies may be viable alternatives to conventional thermal treatments such as blanching and pasteurization (Jiménez-Sánchez et al. 2015; Medina-Meza et al. 2016; Chen & Martynenko 2016a; Chen & Martynenko 2016b; Martynenko et al. 2015; Bruijn et al. 2016; Huang et al. 2007; Lamanauskas et al. 2016; Leong et al. 2016; Oey et al. 2008a). To some extent, these technologies have been exploited commercially for fruits and vegetables. However, application in berry processing is not yet common.

Processing berries into juices results in 70–80% of the target product and 20–30% of press residue, a side-stream consisting of skins, seeds and stems. Freshly pressed berry press residue contains large amount of bioactive phenolic compounds (Cerda et al. 2016; Zheng & Shetty 1998). Currently, the side-stream is used as animal feed, composted, taken as landfill or used for biogas production (O'Shea et al. 2012; Rohm et al. 2015). Viable possibilities for utilizing the press residue could be extraction into phenolic-rich fractions. Macerating treatment with enzymes with targeted activities, microwave heating, pulsed electric field treatment and high pressure processing are potential alternatives to traditional solvent extraction (Costoya et al. 2010; Tomaz et al. 2016; Medina-Meza & Barbosa-Cánovas 2015; Liazid et al. 2011; Corrales et al. 2008). Another possibility for the utilization of the press residue is extrusion cooking for nutritious snacks or breakfast cereals (Khanal et al. 2009a; Khanal et al. 2009b; White et al. 2010).

The first aim of the thesis research was to study the impact of various juice pressing technologies and storage conditions on the chemical composition and sensory properties of black currant (*Ribes nigrum* L.) juices. Special focus was set on the less investigated hydroxycinnamic acids to achieve new knowledge about their molecular structures and behavior during processing and storage. The second aim was to develop extrusion cooking technologies for utilization of the black currant pressing side-streams. The physico-chemical characteristics of the snacks and hedonic response of consumers were studied. The study provides valuable information on the stability and decomposition of berry phenolics during various food processes and storage conditions, and presents possible solutions for sustainable utilization of side-streams.

2 REVIEW OF THE LITERATURE

The literature was approached by searching for processing technologies applied to berries and grapes and their juice pressing side-streams. The processing technologies chosen in the review were those treatment techniques for berries and juices which have been shown to be adequate in reaching the mandated 5-log reduction in most resistant pathogens, as set by the Food and Drug Administration (FDA). In addition, various berry pressing and cooking processes have been reviewed. Wine and other alcohol fermentation processes were excluded, as well as solvent extraction methods. The processing technologies were studied based on their impact on phenolic compounds in the berry-based products. The phenolic compounds of interest were flavonoids (anthocyanins, flavonols and flavan-3-ols), tannins (proanthocyanidins and ellagitannins) and phenolic acids due to their importance for the sensory properties of berry products. However, the impact of the processes on sensory properties has not been dealt within the review.

The berries included in the review are the important garden berries, such as strawberries (*Fragaria × ananassa* Duch.), black currants (*Ribes nigrum* L.), raspberries (*Rubus idaeus* L.), highbush (*Vaccinium corymbosum* L.) and lowbush (*Vaccinium angustifolium* Ait.) blueberries, chokeberries (*Aronia melanocarpa* L.) and blackberries (*Rubus fruticosus* L.), and some well known and commercially important wild bilberries (*Vaccinium myrtillus* L.), raspberries and cranberries (*Vaccinium oxycoccos* L.) and grapes (*Vitis vinifera* L.). Clear and cloudy juices, jams, purées, extracts and extrudates are among the most produced products from berries in the industry, therefore these were also the products chosen for the review (**Figure 1**). Clear juices are produced with supplementary enzymes, which enhance the color of the juice by extracting phenolic compounds from the skin. Cloudy juices are prepared by pressing berries without added enzymes. In the industrial production of purées, whole berries are crushed, sieved and homogenized. Cloudy juices and purées have the native pectins undisrupted, in comparison to clear juices.

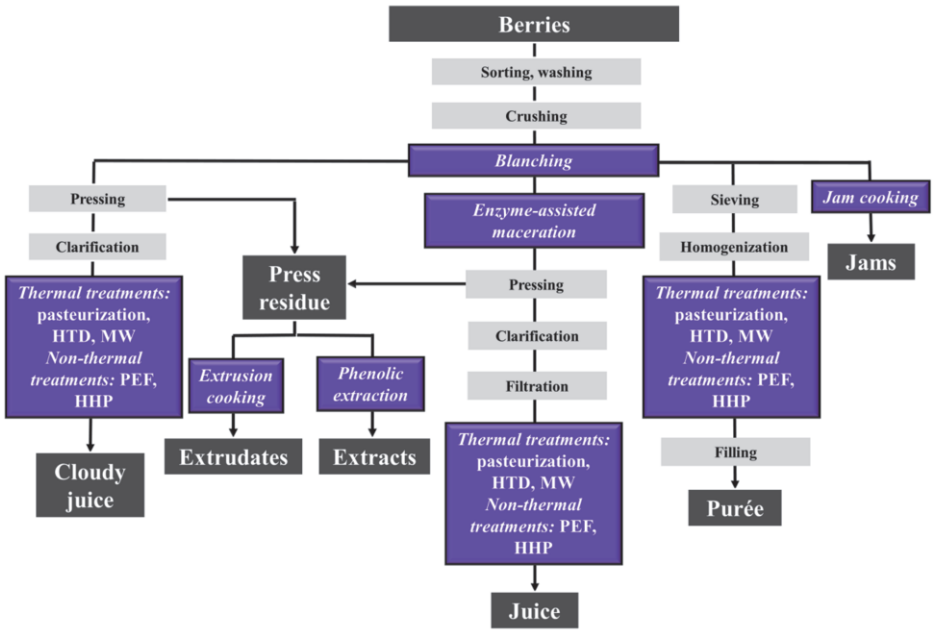

Figure 1. Flow diagram of the thermal and non-thermal process technologies (in violet boxes) studied in the review. Storage stability of the berry products is also evaluated. HTD, hydrothermodynamic; MW, microwave heating; PEF, pulsed electric field; HHP, high hydrostatic pressure.

2.1 Flavonoids, tannins and phenolic acids in berries

Common phenolic groups found in berries are flavonoids, tannins and phenolic acids. The compounds are secondary metabolites present in higher plants. Many of them act as defense compounds against plant pathogens and herbivores. The compounds are often induced by various stress factors, especially in response to UV radiation (El-Seedi et al. 2012). They act as cell wall support materials (Wallace & Fry 1994) and as colorful attractants for birds and insects helping pollination and seed dispersal (Andersen & Markham 2005). In plant tissues, these compounds usually occur as simple substituted phenols, mainly as glycosides, or as complex polymers (Andersen & Markham 2005; Clifford 2000; Macheix et al. 1990).

2.1.1 Flavonoids, tannins and phenolic acids

Flavonoids (anthocyanins, flavonols and flavan-3-ols) posses the characteristic C_6-C_3-C_6 flavone nucleus, consisting of two benzene rings (A- and B-ring)

linked through an oxygen-containing pyran or pyrone ring (C-ring) (**Figure 2**) (Andersen & Markham 2005; Clifford 2000). Anthocyanins are the dominating group of flavonoids found in berries of dark colors (Määttä et al. 2001). Anthocyanins are water-soluble pigments, which contribute to the blue, violet and magenta colors of berries (Clifford 2000). More than twenty anthocyanidins have been characterized, but the most important ones are delphinidin, cyanidin, peonidin, pelargonidin, petunidin, and malvidin (**Figure 2B**) (Kula et al. 2016; Mattila et al. 2016; Prior et al. 2001; Zabetakis et al. 2000). The structural unit is the polyhydroxylated flavylium cation. In nature, the hydroxyl group at C-3 is always glycosylated by e.g. glucose, rhamnose or rutinose (3-*O*-glycosides), and additional glycosylation can occur on hydroxyl groups at C-5, C-7, C-3', C-4' and/or C-5'. Furthermore, the sugar-units can be acylated by various aliphatic or aromatic acids (Iacobucci & Sweeny 1983).

Flavonols (**Figure 2C**) are pale yellow compounds, which usually occur as *O*-glycosides in berries. They have a double bond between C-2 and C-3, a carbonyl group at C-4 and a hydroxyl group at C-3. The preferred site for glycosylation is C-3 (3-*O*-glycosides) and less frequently the C-7 position. Moreover, in flavonols, the sugar moiety may exist in acylated form (Andersen & Markham 2005).

Flavanols (flavan-3-ols) also have a hydroxyl group at C-3 position, but they lack the double bond between C-2 and C-3 and the carbonyl group at C-4 (**Figure 2A**). They possess chiral centers at C-2 and C-3 (Beecher 2003; Treutter et al. 1994). Flavan-3-ols are the building blocks of polymeric condensed tannins (**Figure 2A**). The flavanol monomers are linearly linked to each other by carbon-carbon bonds between C-4 and C-8 of the adjacent monomer, or branched when linked between C-4 and C-6. These types of monomer linkages form polymers of 2 to 50 units (or more). Condensed tannins are also called proanthocyanidins (Bate-Smith 1973; Chung et al. 1998; Fletcher et al. 1977; Yeap Foo & Porter 1980; Wheeler 1979). The compounds are not susceptible to being cleaved by hydrolysis (Wheeler 1979). Proanthocyanidins may be converted into anthocyanins by high temperature treatments under acidic conditions, especially in low moisture environments (Porter et al. 1985).

Ellagitannins and gallotannins are hydrolyzable tannins (**Figure 2A**). At the center of a hydrolyzable tannin molecule there is a carbohydrate, usually glucose, of which OH-groups are partially or totally esterified with phenolic acids. In ellagitannins the phenolic acid is ellagic acid (**Figure 2D**), and in gallotannins gallic acid (**Figure 2A**). These compounds are hydrolyzed by weak acids or weak bases to produce carbohydrate and phenolic acids (Ashok & Upadhyaya 2012; Clifford & Scalbert 2000; Haddock et al. 1982a; Haddock et al. 1982b; Häkkinen et al. 2000b).

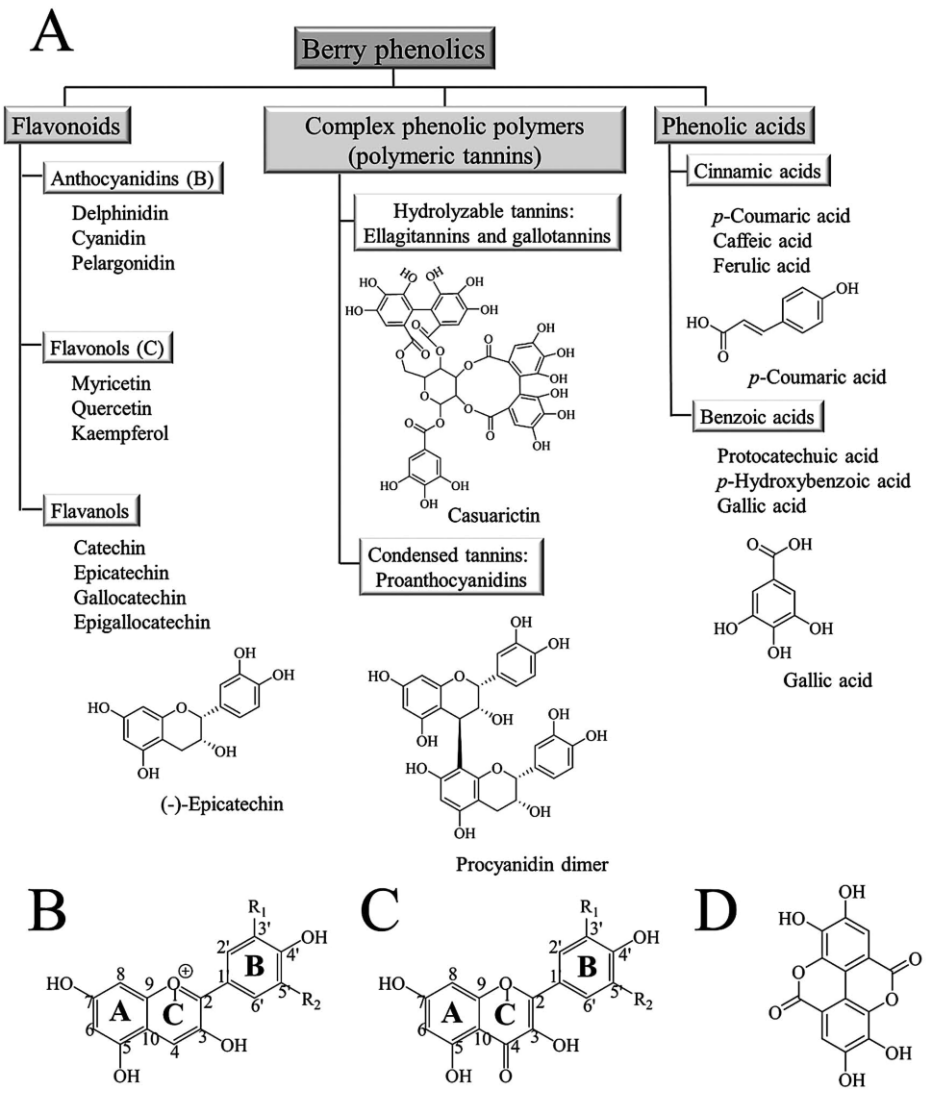

Figure 2. A) Examples of the most common phenolic compounds found in berries (adapted from Puupponen-Pimiä et al. 2005). B) Chemical structures of anthocyanidins: delphinidin, R_1=OH, R_2=OH; cyanidin, R_1=OH, R_2=H; pelargonidin, R_1=H, R_2=H; peonidin, R_1=OCH_3, R_2=H; petunidin, R_1=OCH_3, R_2=OH and malvidin, R_1=OCH_3, R_2=OCH_3. C) Chemical structures of flavonols: kaempferol, R_1=H, R_2=H; quercetin, R_1=OH, R_2=H and myricetin, R_1=OH, R_2=OH. D) Chemical structure of ellagic acid.

Phenolic acids present in plants are derivatives of hydroxybenzoic and hydroxycinnamic acids. The most commonly occuring hydroxybenzoic acids are *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acid. They may be

conjugated with sugars or organic acids as well as bound to cell wall fractions (Schuster & Herrmann 1985; Strack 1997). The most common hydroxycinnamic acids (HCAs) found in nature are coumaric (**Figure 2A**) and caffeic acids with one and two OH groups, respectively, and ferulic acids with one OH group and one OCH_3 group in the aromatic ring. HCAs exist commonly as *O*-acylglucoses, *O*-glucosides or as *O*-acylquinic acids (El-Seedi et al. 2012). In nature, the acids rarely exist in free form (Manach et al. 2004; Wilkes et al. 2014). The *E*-isomer of HCAs is predominant and found to be more stable (Turner et al. 1993). The *Z*-isomer exists as a result of sun- and UV-light activating the defense mechanism of plants (Chen et al. 2011).

2.1.2 Cell wall structures and the distribution of phenolic compounds in berries

Plant cell wall material (**Figure 3**) consists of various polysaccharides, i.e. pectins, hemicellulose, cellulose and cutin. The middle lamella (**Figure 3A**) binds cells together and is mainly composed of pectin. The structure of pectin includes acidic polymers such as homogalaturonans and rhamnogalacturonans attached with neutral polymers like arabinans, galactans and arabinogalactans (Albersheim et al. 1996). The berry pulp/flesh is especially high in pectin. The riper the berries, the less pectin they contain (Hilz et al. 2005). The primary cell wall is comprised of cellulose-xyloglucan framework, and is embedded in a matrix of pectin polysaccharides. It also contains structural proteins (Bidlack et al. 1992). The other polysaccharides (hemicellulose, cellulose and cutin) are mainly located in the skin and seed coat of berries (Hilz et al. 2005; Järvinen 2010).

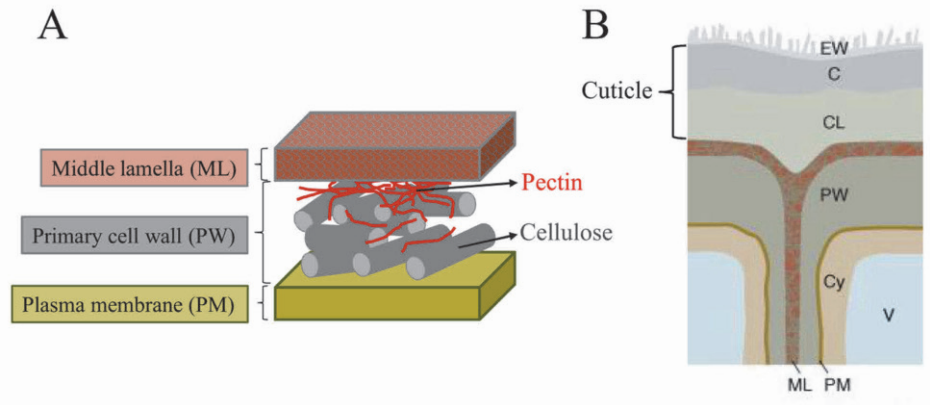

Figure 3. A) Plant cell wall structures and B) cuticle. EW, epicuticular waxes; C, cuticle proper (cutin); CL, cuticular layer; Cy, cytoplasm; V, vacuole. Adapted from Dal Magro et al. 2016 and reproduced with permission (Pollard et al. 2008). Copyright 2008 Elsevier.

The epidermal cells of plants are covered with an extracellular cuticular layer (**Figure 3B**), basically composed of a cutin matrix with waxes embedded in (cuticular layer) and deposited on the surface of the matrix (epicuticular waxes). Cutin is the main structural and protective polymer in the berry surfaces. The cutin consists of esters of hydroxyl and/or epoxy fatty acids (Heredia 2003; Kolattukudy 1996; Pollard et al. 2008; Walton 1990). Cuticular waxes is a general term to describe complex mixtures of homologue series of long-chain aliphatics, such as alkanes, aldehydes, alcohols, esters and fatty acids, with the addition of varying proportions of cyclic compounds such as hydroxycinnamic acid derivatives and triterpenoids (Bianchi 1995; Heredia 2003; Radler & Horn 1965; Walton 1990). Cuticular polysaccharides link the cuticular layer to the epidermal cells, and are responsible for the elasticity and stiffness of the cuticle (López-Casado et al. 2007) and for providing the varying mechanical properties of berries (Järvinen 2010; López-Casado et al. 2007).

In berries and grapes, phenolic compounds can be classified as cell wall phenolics bound to polysaccharides by hydrophobic interactions and hydrogen bonds, and non cell wall phenolics, encompassing phenolics located in the vacuoles of the berry cell, within the cell cytoplasm or associated with the cell nucleus. The majority of phenolic compounds are located in the skin of berries and grapes due to biosynthesis stimulated by light (Cortell & Kennedy 2006; Laaksonen et al. 2010; Pinelo et al. 2006; Sandell et al. 2009).

The phenolic composition and distribution varies among berries (Mattila et al. 2006). Anthocyanins are mainly located in the vacuoles of berries (**Figure 3B**) (Tanaka et al. 2008). In some berries and red grapes, the anthocyanins are mainly distributed in the skin, while in other berries anthocyanins appear throughout the fruit (Lee & Wrolstad 2004; Monagas et al. 2006; Pinelo et al. 2006; Sablani et al. 2010). Grape skin contains the highest amounts of tannins compared to other grape fractions (Souquet et al. 1996). Most of the proanthocyanidins are present in the berry press residue (Khanal et al. 2009a; Khanal et al. 2009b). Ellagitannins occur in particularly high concentration in strawberry achenes (Aaby et al. 2005). Phenolic acids are esterified with pectins and arabinoxylans constructing the berry cell wall. The content of the phenolic acids decreases as the berry ripens (D'Archivio et al. 2007). Degradation of cell wall polysaccharides is a fundamental step to improving the mass transfer and release of phenolics from the skin into juice during berry pressing.

2.2 Stability of phenolic compounds

Factors influencing the stability of phenolic compounds during processing and storage of berry products are, among other things, the type of process and the process parameters, the matrix of the berry products and storage conditions. In addition, the chemical structures of the aglycones and substituents influence the stability of phenolic compounds. Anthocyanins are especially vulnerable during processing and storage. Therefore, the factors influencing the stability of anthocyanins and the degradation mechanisms are reviewed in more details compared to other phenolic compounds.

pH. Stability of anthocyanidin is pH dependent, since protonation and deprotonation of the molecule changes and breaks the conjugated double bond system and its resonance, changing also the color of the molecule. Anthocyanidins are predominantly in the form of red flavylium cations in acidic environments (pH ≤ 2) (**Figure 4A**). At a higher pH (pH 2–4), the flavylium cation is rapidly hydrated, resulting in the formation of a colorless carbinol pseudobase (**Figure 4C**). The carbinol pseudobase can be converted over time into an open ring structure, i.e. the chalcone pseudobase (**Figure 4D**). In an aqueous medium, including foods, a blue quinonoidal base also exists (**Figure 4B**) (Kalt et al. 2000; Patras et al. 2010). These forms vary in their susceptibility to oxidative degradation and complex formation and reactions with other phenols, metals, amino acids and proteins (Friedman & Jürgens 2000; Lapidot et al. 1999).

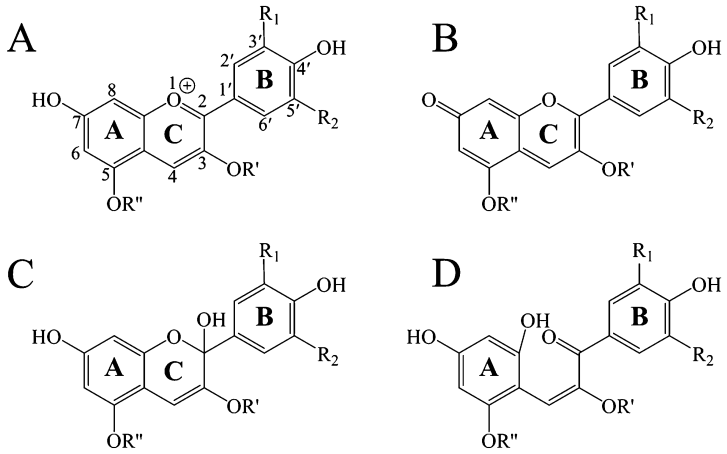

Figure 4. Anthocyanidin structures present in aqueous acidic solution. A) Flavylium cation (red), B) quinonoidal base (blue), C) carbinol pseudobase (colorless) and D) chalcone (colorless) (adapted from Schwartz et al. 2008).

According to Friedman and Jürgens (2000), phenolic compounds, which were found stable as regards changes in the pH in the model solutions were rutin, (–)-catechin, (–)-epigallocatechin and ferulic acid. These compounds were resistant to major pH-induced degradation shown as reversible UV-spectral transformations. The pH-stability of the phenolic compounds may derive from relative resonance stabilization of phenoxide ions and their inability to form quinone oxidation products (Friedman & Jürgens 2000; Mataga & Kubota 1970). The monocyclic phenolic compounds of caffeic, chlorogenic and gallic acids were not stable at a high pH in the model solutions, indicating that the presence of more than one OH-group in the benzene ring labilizes the system (Friedman & Jürgens 2000).

Complex formation. Co-pigmentation has been suggested as the main color stabilizing mechanism in plants. Types of co-pigmentation reactions are self-association between anthocyanins, and intramolecular and intermolecular co-pigmentation (Brouillard 1982). Water molecule hydrates the pyrylium cation, leading to conversion of the flavylium ion into the colorless pseudobase and chalcone forms, consequently resulting in the loss of color. Co-pigment protects the colored flavylium cation from the nucleophilic attack by water molecule (Brouillard 1981; Kopjar & Piližota 2009).

In intramolecular co-pigmentation, the co-pigment (organic acid, aromatic acyl group, flavonoid or a combination thereof) is covalently linked to the anthocyanin chromophore (Brouillard 1982; Rein 2005; Shikov et al. 2012; Wilska-Jeszka & Korzuchowska 1996). The flavylium ion is positively charged, possessing electron-poor aromatic ring π-systems. Thus, the flavylium ion can easily participate in the formation of a charge transfer complex with electron-rich aromatic rings of co-pigments. Electrons are transferred from the electron-rich aromatic ring to the electron-poor aromatic ring. The formation of $\pi - \pi$ complex leads to altered spectral properties of the flavylium ion, observed as a bathochromic shift (the absorption spectra moves towards longer wavelengths), resulting in the more bluish hues of anthocyanins (Mazza & Brouillard 1987; Türkyılmaz et al. 2012; Asen et al. 1972). Co-pigmentation can also be observed as a hyperchromic effect (increase in color intensity), in which the red/magenta hues of an anthocyanidin are enhanced (Rein 2005).

Acylated anthocyanins are naturally occurring complexes formed by intramolecular co-pigmentation, where anthocyanidins are co-pigmented e.g. with aromatic acids such as *p*-coumaric, ferulic and sinapic acids. These types of co-pigments are most often bound to the C-3 sugar, esterified to the 6-OH group of the sugar molecules, or less frequently to the 4-OH group of the sugars (Kammerer et al. 2003; Kammerer et al. 2004). Aromatic acids as co-pigments are less frequently present in berries (Kähkönen et al. 2003; Seeram

et al. 2006), but are present in grapes (García-Beneytez et al. 2002; García-Beneytez et al. 2003).

In red wine, bicyclic anthocyanin-tannin/flavan-3-ol co-pigmentation was found to form through direct condensation reactions, where the C-4 position of the anthocyanidin reacted with flavanol monomer at the C-6 or C-8 position (Remy et al. 2000). Condensation reactions resulted in loss of color or formation of brown colors if the conjugated double bond system was disrupted as a result of hydration of the pyrylium ring (Jackman & Smith 1996; Osawa 1982; Remy et al. 2000). Association with metals is also a form of intramolecular co-pigmentation. Anthocyanins form complexes with metals such as iron, copper, magnesium and aluminum attached to the *ortho*-hydroxy groups of the anthocyanidin B-ring (Brouillard 1982; Castañeda-Ovando et al. 2009; Cavalcanti et al. 2011). The intramolecular co-pigment effect is more efficient in protecting anthocyanidins against water nucleophilic addition, than the intermolecular effect (Brouillard 1981; Kopjar & Piližota 2009).

Intermolecular co-pigmentation is an interaction between a colored anthocyanin and a colorless co-pigment, such as flavonol or flavone glycosides or other phenolic compounds (Brouillard 1982), which is not bound covalently to the anthocyanin molecule (Brouillard 1983). Hydrophobic effects, ionic interactions and van der Waals forces have been suggested as the main mechanistic driving forces for intermolecular co-pigmentation (Asen et al. 1972; Dangles et al. 1993). Intermolecular co-pigmentation may occur with both the flavylium cation and the quinonoidal base forms of anthocyanins (Asen et al. 1972). Co-pigmentation of anthocyanins with polysaccharides and proteins is also possible (Friedman & Jürgens 2000; Jackman & Smith 1996; Lapidot et al. 1999; Osawa 1982).

Adducts. Anthocyanin adducts, pyranoanthocyanins, were found in strawberry and raspberry juices after addition of cinnamic acids to the berry juices. The pyranoanthocyanins were formed between the anthocyanidins and cinnamic acids, linked at the C-4 and 5-OH positions of the anthocyanidin. These new adducts enhanced and stabilized the color of the berry juices (Rein et al. 2005).

Degradation of anthocyanins is mostly caused by oxidation, cleavage of covalent bonds, or enhanced oxidation due to thermal processing (Kalt et al. 2000; Nebesky et al. 1949). Oxygen degrades anthocyanins either through a direct oxidative mechanism and/or through the action of oxidizing enzymes (Jackman et al. 1987a).

Oxidizing enzymes. Oxidizing native enzymes, such as polyphenol oxidase (PPO, EC 1.14.18.1) and peroxidase (POD, EC 1.11.1.7), are mainly located in the berry cytoplasm. During crushing of berries the enzymes come into contact with anthocyanins and other phenolic compounds located predominantly in the

vacuoles. In the presence of oxygen, PPO catalyzes the oxidation of chlorogenic acid and catechin into corresponding *ortho*-quinones, which subsequently react with anthocyanins producing brown pigments (Jiménez & García-Carmona 1999; Kader et al. 1997; Kader et al. 1998; Lee et al. 2002; Wesche-Ebeling & Montgomery 1990). Anthocyanidins with an *ortho*-diphenolic B-ring are degraded by *ortho*-quinones (Kader et al. 1997; Sarni et al. 1995). Anthocyanidins without *ortho*-diphenolic B-ring can form adducts with the *ortho*-quinones (Kalt et al. 2000). Incomplete deactivation of PPO and POD during processing would significantly affect storage stability of anthocyanins and other phenolic compounds in berry products (Chisari et al. 2007; López-Serrano & Ros Barceló 2002). Flavonols were found less susceptible to native enzymatic degradation than anthocyanins (Skrede et al. 2000).

Thermal degradation. In the pH range of most berry juices (pH 2–4), equilibrium exists between four forms of anthocyanin molecules: flavylium cations, quinonoidal bases, carbinol pseudobases and chalcones. Increasing the temperature reverses the equilibrium towards formation of chalcones (colorless), diminishing the quinoidal, flavylium and carbinol pseudobase species (Brouillard 1982). These chalcones can be degraded to brown-colored polymeric structures when exposed to high temperatures for a prolonged time, causing a decrease in the anthocyanin content of berry juices (Dangles & Brouillard 1992; Fulcrand et al. 1998). Heating at 90 °C, the color of the anthocyanin model system changed progressively from red to pale red, and from a colorless to yellow to brown (Tsai & Huang 2004).

The thermal degradation pathway of anthocyanins seemed to be both temperature and pH-dependent. Heat treatment (at 95 °C for several hours) of anthocyanin isolates at pH 1 deglycosylated the structures and the corresponding anthocyanidins degraded further to hydroxybenzoic acids (**Figure 5**). The B-ring of cyanidin degraded into protocatechuic acid, and of pelargonidin into 4-hydroxybenzoic acid, whereas the A-ring decomposed into phloroglucinaldehyde (Sadilova et al. 2006). At pH 3.5, the degradation occurred through chalcone glycoside formation, following the deglycosylation to yield chalcone, and resulting in the corresponding hydroxybenzoic acids (Sadilova et al. 2007).

Thermal degradation of anthocyanins usually follows first-order reaction kinetics as shown in the equation (1) under isothermal heating (Garzon & Wrolstad 2002; Kechinski et al. 2010; Patras et al. 2010). The rate of degradation of anthocyanins is highly dependent on the pigment aglycone, the sugar substituent and possible acylation.

$$C_t = C_0 \times exp(-k \times t) \quad (1)$$

Where C_t is the anthocyanin concentration at time t, C_0 is the initial concentration and k is the rate constant. Exposure to UV and visible light may further increase the rate of thermal degradation of anthocyanins (Jackman et al. 1987b; Kearsley & Rodriguez 1981).

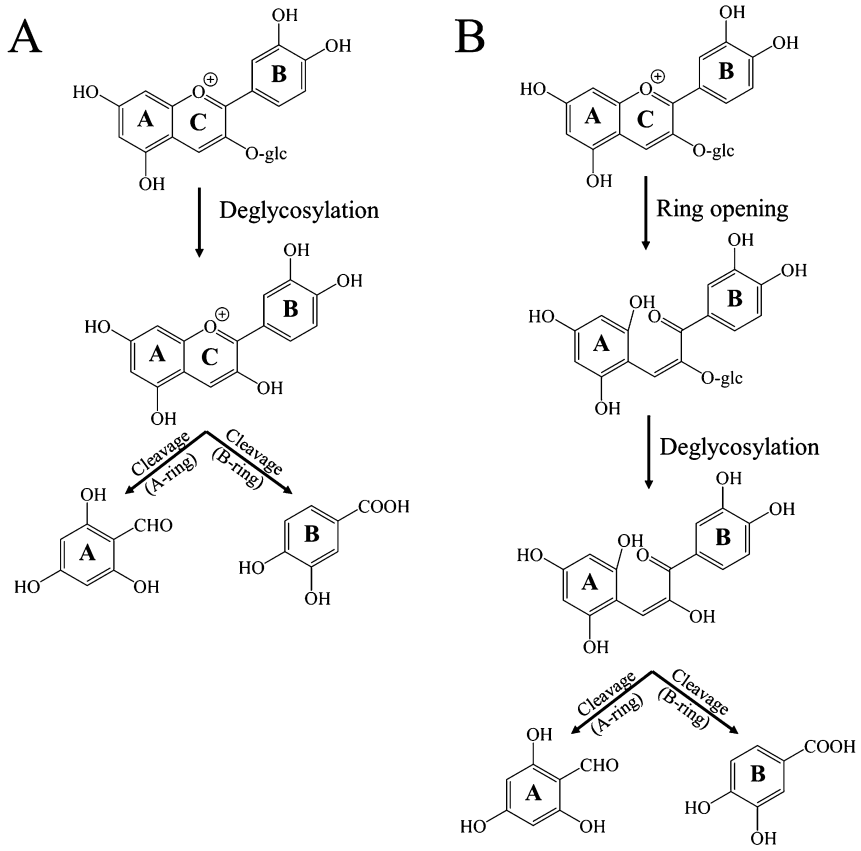

Figure 5. Thermal degradation (at 95 °C) of cyanidin glucoside A) at pH 1 and B) at pH 3.5 isolated from strawberry, elderberry and black carrot (adapted from Sadilova et al. 2006 and Sadilova et al. 2007).

For flavonols, tannins and phenolic acids, heat seems to lead to spontaneous hydrolysis of bound compounds into free forms. Flavonols are generally better retained during thermal treatments than anthocyanins. However, significant quantities of flavonol aglycones have been reported in thermally processed berry products. These aglycones are likely derived from hydrolysis of their corresponding glycosides (Amakura et al. 2000; Marszałek et al. 2015; Odriozola-Serrano et al. 2008b; Vvedenskaya et al. 2004; Wilkes et al. 2014). Proanthocyanidin oligomers of high degree of polymerization (DP) were found to decrease during heat processing, explained by their conversion into

proanthocyanidin monomers, dimers, and trimers (Capanoglu et al. 2013; Fuleki & Ricardo-da-Silva 2003; White et al. 2010; Khanal et al. 2009a; Khanal et al. 2009b; Brownmiller et al. 2009).

Levels of free ellagic acid have been found to increase during thermal processes (Odriozola-Serrano et al. 2008b; Rodriguez-Mateos et al. 2014; Wilkes et al. 2014). The increase is probably due to the release of hexahydroxydiphenic acid from ellagitannins and transformation to ellagic acid (Odriozola-Serrano et al. 2008b; Zafrilla et al. 2001). Phenolic acids seldom exist in free forms unless the derivatives have undergone processing or storage resulting in hydrolysis (Manach et al. 2004; Wilkes et al. 2014). Storage at ambient room temperatures has been found to lead to similar decomposition of phenolic compounds as a thermal treatment (Bimpilas et al. 2015; Kallithraka et al. 2009; Wilkes et al. 2014).

Polymerization. During processing and storage monomeric forms of anthocyanins have been found to transform into polymeric forms resulting in colorless derivatives and subsequently insoluble brown pigments. The increased polymeric color has been suggested to indicate the formation of anthocyanin-tannin (Brownmiller et al. 2008; Hager et al. 2008; Wilkes et al. 2014) or anthocyanin-(poly)flavan-3-ol polymers (Reed et al. 2005). The polymerization is further enhanced by high process and storage temperatures (Brownmiller et al. 2008; Hager et al. 2008; Howard et al. 2010; Poiana et al. 2012; Wilkes et al. 2014). Juices with a lower pH were found to result in lower levels of polymeric pigments (Kalt et al. 2000).

The formation of polymerized color can be measured as a percentage of polymeric color, which is indicative of a tannin/flavanol molecule attached to the anthocyanin molecule at C-4, and is generally considered as an index for anthocyanin degradation (Giusti & Wrolstad 2001). An illustration of the precise structure of the anthocyanin-tannin/flavanol polymer formed in the berry matrix could not be found from the literature. However, similar types of anthocyanin-tannin/catechin polymers have been detected in aging wines and model solutions. These polymers were formed *via* condensation, yielding colorful or colorless 4-substituted flav-2-enes pigments, often mediated by acetaldehyde. This type of polymerization reaction has also been described as co-pigmentation (Escribano-Bailón et al. 1996; Remy et al. 2000). This hinders the clear separation between polymerization and co-pigmentation reactions. However, in most cases, co-pigmentation results in stabilization of the flavylium cation in the complex, whereas, polymerization reactions seem to take place with other forms of anthocyanidins than the flavylium cation, and result in colorless or brownish pigments.

Pectins. Native and additional pectins enhance the stability of anthocyanins due to the formation of hydrocolloidal suspensions. These suspensions create

similar surroundings to encapsulation, involving closure of anthocyanin molecules in the pectin gel (Hartmann et al. 2008; Holzwarth et al. 2013; Maier et al. 2009; Poiana et al. 2012).

Ascorbic acid and sugar. Ascorbic acid, additional sugar, as well as their degradation products may decrease the stability of anthocyanins (Meschter 1953; Nikkhah et al. 2007). At temperatures higher than refrigeration, oxidative degradation of ascorbic acid can accelerate the production of many reactive degradation products, such as hydrogen peroxide. The reactive degradation products probably interact with anthocyanins, causing a cleavage of the pyrylium ring (García-Viguera & Bridle 1999; Sun et al. 2009), and enhance the polymeric pigment formation (Poei-Langston & Wrolstad 1981). In addition, degradation of ascorbic acid may lead to non-enzymatic browning of berry products *via* a reaction between α-amino groups and reducing sugars in response to thermal treatment and oxygen; this is called the well-known Maillard reaction (Cao et al. 2011; Cao et al. 2012; Stintzing & Carle 2004; Wrolstad et al. 2005). On the other hand, high sugar concentrations in the berry products may enhance the stability of anthocyanin due to reduced water activity (Castañeda-Ovando et al. 2009).

2.3 Effect of thermal processing on phenolic compounds in berry products

Traditional preservation methods inactivate microbes and native oxidizing enzymes to ensure the stability of the color and microbiological safety of the products. These methods are based on the addition of heat, and depend on the pH of the product as well as the desired shelf-life. Traditional heat treatments, such as blanching and pasteurization degrade the phenolic compounds, especially the anthocyanins, of berries and berry-based products. There is a real need for processing technologies, which allow a better retention of these phenolic compounds. Hydrothermodynamic technology and microwave heating may be valuable technologies for a better retention of the phenolic compounds in berry-based products in comparison to traditional thermal treatments.

2.3.1 Blanching

Blanching is a thermal treatment (e.g. 75–95 °C for 1–10 min) conducted with water, steam, vacuum-steam or hot-air. Blanching is executed prior to freezing, drying, or after the crushing of berries. The purpose is to inactivate oxidizing native enzymes such as PPO and POD, and thus, stabilize the color of berry products. Inactivation of PPO requires approximately 3–4 min blanching at temperatures above 85 °C. POD is already inactivated at 70 °C for 5 min

(Carranza-Concha et al. 2012; Chisari et al. 2007; Jiménez & García-Carmona 1999; Kader et al. 1997; Kader et al. 1998; Skrede et al. 2000; Terefe et al. 2010). The varying activities of native enzymes, along with the varying pH of the berries or sufficiency of substrates, result in different impacts of blanching in different berry species (Kader et al. 1997; White et al. 2011).

Anthocyanins. Blanching of berries and grapes at 95–100°C for 1–5 min was found to increase (by 50%) the retention, have no impact, or cause significant losses (60%) in monomeric anthocyanins in the juices pressed with blanched berries. The results were compared to fresh berries of juices pressed with unblanched berries (Brownmiller et al. 2008; Rio Segade et al. 2014; Rossi et al. 2003; Sablani et al. 2010; White et al. 2011; Wilkes et al. 2014). The increase might have derived from the heat-induced skin disruption releasing anthocyanins from the vacuoles (Sablani et al. 2010).

The impact of blanching seemed to be more correlated with the berry species rather than the process parameters (Brownmiller et al. 2008; Rio Segade et al. 2014; Rossi et al. 2003; Sablani et al. 2010; White et al. 2011; Wilkes et al. 2014). The varying impacts of blanching on berries and grapes may be due to differences in the distribution of anthocyanins in the skin and in the pulp of berries from different species. In some berries and red grapes, the anthocyanins are mainly distributed in the skin, while in other berries anthocyanins appear throughout the fruit (Lee & Wrolstad 2004; Monagas et al. 2006; Pinelo et al. 2006; Sablani et al. 2010). Anthocyanins of blueberries seemed to be especially vulnerable to blanching. The anthocyanins of blueberries are mainly concentrated in the skin (Määttä-Riihinen et al. 2004), which get more efficiently in contact with the heat.

Different structures of anthocyanins lead to different heat sensitivities during blanching. Methoxylation of the acyl moiety improved the structural integrity in relation to heat. The type of sugar attached to the aglycone affected the stability of the compounds. Glucosides of anthocyanidins remained stable, but galactosides decreased by 20%. Pentosides were more susceptible to degradation during the blanching than hexosides (White et al. 2011; Wilkes et al. 2014). Polymeric color values decreased in response to blanching (at 95 °C for 3 min) of chokeberries, indicating cleaveges of anthocyanin-tannin/flavanol polymers (Wilkes et al. 2014).

Flavonols. Blanching (at 95 °C for 3 min) of chokeberries and cranberries significantly increased the content of free myricetin and quercetin. This increase was coupled with slight decreases in the amounts of flavonol glycosides, indicating deglycosylation of the glycosides into aglycones as a result of processing. In addition, increased contents of flavonol glycosides were observed, indicating that the heat treatment enhanced the flavonol extraction from the berry skin (White et al. 2011; Wilkes et al. 2014). Flavonols seemed

to be relatively more heat stabile during blanching, compared to anthocyanins (White et al. 2011).

Tannins. Blueberries, chokeberries and cranberries were blanched at 95 °C for 3 min. The content of total procyanidins decreased by 20% after the blanching of blueberries compared to fresh berries (Brownmiller et al. 2009). The content of total proanthocyanidins remained stable in the juices prepared from blanched chokeberries (Wilkes et al. 2014). Blanching of cranberries increased the content of total procyanidin oligomers with the degree of polymerization (DP) DP1–DP6 and polymers with DP > 10 in the juice (White et al. 2011). The increase might be explained by their release from cell wall polysaccharides as a result of enhanced tissue softening after heating. In blueberries, the level of mono- and trimers of procyanidins decreased by 20%, whereas dimers of procyanidins increased by 40%, possibly due to conversion of oligomers into dimers (Brownmiller et al. 2009). Blanching enhanced the extractability of ellagitannins from blackberries (Hager et al. 2010) probably due to heat-induced tissue softening.

Phenolic acids. The contents of chokeberry protocatechuic acid and phloroglucinaldehyde increased up to 400% upon blanching treatment. These hydroxybenzoic acids were most probably anthocyanin degradation products following the proposed pH-dependent thermal degradation of cyanidin at pH 3.5, which is close to the pH of chokeberries (**Figure 5B**). The level of chlorogenic acid increased by 10%, but the level of neochlorogenic acid did not change (Wilkes et al. 2014).

2.3.2 Pasteurization

Pasteurization is a heat treatment (70–100 °C) used to reach a 5-log pathogen reduction in berry products (Howard et al. 2012). It is a short-term heat treatment like blanching. Pasteurization is most often applied to the final product.

Anthocyanins. Pasteurization (at 90–107 °C for 1–15 min) of berry and grape juices resulted in marked or minor losses of monomeric anthocyanins by 5–70%, compared to unpasteurized juices, depending on the berry and process parameters (Brownmiller et al. 2008; Capanoglu et al. 2013; Lee et al. 2002; Wilkes et al. 2014). A higher stability of anthocyanins was achieved by lowering the temperature and shortening the heating time to 85 °C for 5 seconds (Hartmann et al. 2008; Kechinski et al. 2010). When unblanched blueberry juice was pasteurized, an increase in anthocyanin content was observed, probably because the pigment degrading PPO and POD enzymes remained activite until pasteurization (Skrede et al. 2000).

Delphinidin glycosides were the more unstable of the anthocyanins in blueberry juice during pasteurization, compared to glycosides of cyanidins, petunidins, peonidins and malvidins. The order of lability seemed to be dependent on the amount of hydroxy groups in the B-ring. Malvidin glycosides with the highest stability were found to even increase during pasteurization, owing to the two adjacent methoxy substituents (Skrede et al. 2000). As was observed during blanching, pentosides of anthocyanidins were more susceptible to degradation than hexosides during pasteurization (White et al. 2011; Wilkes et al. 2014). Polymeric color increased during pasteurization, indicating brown color polymer formation between anthocyanin and epicatechins, proanthocyanidins or tannins (Hager e al. 2008; Wilkes et al. 2014). The formation of polymers might have explained some of the losses in monomeric anthocyanins (Wilkes et al. 2014).

Flavonols. During pasteurization (at 90–95 °C for 10 min), levels of total flavonols decreased by 15% or remained stable in the berry juices, depending on the berry species (White et al. 2011; Wilkes et al. 2014). Flavonol glycosides either decreased (up to 20%) or remained stable, accompanied by increase in free aglycones (up to 60%) in chokeberry, blueberry and grape juices, compared to unpasteurized juice (Capanoglu et al. 2013; Skrede et al. 2000; Wilkes et al. 2014).

Tannins. Pasteurization (at 90 °C, no time recorded) resulted in significant losses of total procyanidins, with 40–80% decrease in mono-, di-, tri- and tetramers and up to 90% decrease in penta-, hexa-, and heptamers in blueberry juices (Brownmiller et al. 2009). In grape juices, pasteurization (at 85–107 °C) resulted in depolymerization of higher oligomeric and polymeric procyanidins into dimers and trimers (Capanoglu et al. 2013; Fuleki & Ricardo-da-Silva 2003). Pasteurization (at 90 °C for 10 min) had no effect on the procyanidin oligomers in cranberry juices (White et al. 2011) and the total ellagitannins in blackberry juices (Hager et al. 2010), indicating the heat stability of these compounds in these berry species. Pasteurization increased the content of flavan-3-ols in cold-pressed (at 0 °C) grape juices and decreased the content in thermally (at 40–60 °C) pressed juices (Capanoglu et al. 2013; Fuleki & Ricardo-da-Silva 2003).

Phenolic acids. Contents of protocatechuic acid and phloroglucinaldehyde increased from pasteurization (at 90 °C for 10 min) by 30% in chokeberry juice partly due to the degradation of anthocyanins into hydroxybenzoic acids. Neochlorogenic acid showed a 10% loss from pasteurization, whereas the content of chlorogenic acid was minimally affected in chokeberry juices (Wilkes et al. 2014). Chlorogenic acid suffered marked losses in blueberry juice (Lee et al. 2002), even though the temperature (90 °C) was the same and the treatment time was shorter (90 s vs. 10 min) compared to the study of

chokeberry juice. In contrast, pasteurization (at 90 °C for 1 min) was also found to lead to a higher content of chlorogenic acid in blueberry juice (Skrede et al. 2000).

2.3.3 Hydrothemodynamic processing

Hydrothermodynamic (HTD) technology is based on cavitation, i.e. creation of microscopic bubbles in liquid foods. In HTD, a liquid product circulates through an obstacle (cavitator), which causes turbulence and multiphase cavitation bubbles. The bubbles are unstable and collapse immediately after passing through the active area of the cavitator. This results in rapid localized changes in pressure, creating shear forces and releasing energy for crushing and homogenizing solid particles. Shear forces cause the rupture of plant tissues, improving the release of phenolic compounds from the berry skin into the berry products (Chen & Martynenko 2016b; Knorr et al. 2002; Leighton 1998; Martynenko et al. 2015).

The cavitation energy can be converted into pasteurization temperatures under certain conditions. The process operates with a limited exposure to oxygen in a closed system, thus reducing polyphenolic oxidization (Chen & Martynenko 2016b; Martynenko et al. 2015). HTD treatment can be used for berry purée production with a 100% mass recovery, simultaneous pasteurization, and crushing and homogenizing of the product in a single-unit operation (Chen & Martynenko 2016b; Martynenko et al. 2015; Satanina et al. 2014). The cavitation of the HTD process, along with the temperature, might further enhance the deactivation of PPO and POD enzymes by affecting the protein structure of these enzymes (Martynenko et al. 2015).

Anthocyanins. HTD processing of berries into purées retained the content of anthocyanins to a higher extent when compared to the levels of anthocyanins in corresponding commercial pasteurized berry products (Chen & Martynenko 2016b; Martynenko & Chen 2016; Satanina et al. 2014; White et al. 2011). This higher retention with HTD was likely due to the closed batch processing with a low presence of oxygen (Jackman et al. 1987b; Kalt et al. 2000). The lower content of anthocyanins in commercial products might possibly have been derived from their long storage on the market shelves. The degree of retention of anthocyanins in the HTD process was highly dependent on the treatment temperature. The anthocyanin content of cranberry and blueberry HTD purées decreased linearly with increasing temperatures and holding times. At lower temperatures (below 65 °C), HTD processing did not have a significant effect on the anthocyanin content (Chen & Martynenko 2016b; Martynenko & Chen 2016). A fairly significant drop in anthocyanin content of HTD processed fresh blueberries was observed (Martynenko et al. 2015),

probably due to the release of anthocyanin degrading PPO enzyme during homogenization (Lee et al. 2002).

Anthocyanin degradation was followed by increased polymeric color. Polymeric color formation correlated positively with temperature and holding time, especially at temperatures in the range of 80–105 °C (Chen & Martynenko 2016b; Martynenko & Chen 2016). Polymeric color values were significantly lower in HTD processed blueberry purée compared to the corresponding commercial products (Satanina et al. 2014).

Tannins. HTD treatment at 95 °C for 5 min increased the total proanthocyanidins by 30% in cranberry purées (Chen & Martynenko 2016b). An explanation for this could be the release of proanthocyanidins from the fiber matrix and seeds as the result of finer crushing with longer heating, as was found in the case of cranberries and blueberries (Chen & Martynenko 2016b; Martynenko et al. 2015; White et al. 2011).

2.3.4 Microwave heating

Microwave heating (dielectric heating) technique has been applied in the food industry for pasteurization, sterilization and extraction (Bruijn et al. 2016; Huang et al. 2007; Marszałek et al. 2015; Picouet et al. 2009; Simić et al. 2016), and has been extensively studied in the drying of grapes (Bingol et al. 2008; Clary et al. 2005; Liazid et al. 2011; Tulasidas et al. 1993; Tulasidas et al. 1996; Tulasidas et al. 1997). Microwave heating enables a rapid rise in temperature, controllable heat distribution, the possibility of cooling the product in the flow, and less heat loss to the environment. The radiant energy of microwaves heats the product directly, which makes it possible to treat higher-density berry products, such as strawberry purées and products with solid particles (Marszałek et al. 2015). Microwave treatment also partially inactivates PPO and POD enzymes (Marszałek et al. 2015).

Anthocyanins. Microwave processing (at 90–135 °C for 7s) retained the anthocyanin composition better, compared to the conventional thermal treatment (at 100 °C for 1–5 h) of chokeberry and honeysuckle juices. The anthocyanins of honeysuckle juice were more thermally stable, compared to those of chokeberry juices (Piasek et al. 2011). Microwave heating at 120 °C for 10 s retained the content of anthocyanins by a 20% higher level compared to pasteurization (90 °C for 15 min) of strawberry purée. Microwave heating did not cause significant changes to the color of the purée, whereas pasteurization did (Marszałek et al. 2015). A temperature of a 100 °C was found to be the maximum for use in developing the microwave assisted extraction method for maximal anthocyanin recovery from grape skin. There

was no clear difference between glucosides and the acyl derivatives during the extraction of anthocyanins (Liazid et al. 2011).

Flavonols and tannins. Microwave heating (at 90–135 °C for 7s) did not produce quercetin aglycones as did pasteurization (at 100 °C for 1–5 h) of chokeberry and honeysuckle juices, with the content increasing as the pasteurization time increased (Piasek et al. 2011). The content of procyanidin dimers and gallic acid decreased to trace amounts in choke berry juice during both conventional thermal (at 100 °C) and microwave heating (at 90–135 °C) (Piasek et al. 2011).

Phenolic acids. The content of protocatechuic acid increased to a higher extent during conventional thermal treatment at 100 °C, compared to microwave heating at 90–135 °C in aronia juice (Piasek et al. 2011). The higher increase probably resulted from a higher degradation of cyanidin (Sadilova et al. 2006; Sadilova et al. 2007). The content of 3-*O*-caffeoylquinic acid was lower and the content of 5-*O*-caffeoylquinic acid was higher after microwave heating of choke berry juice, compared to conventional thermally processed juice (Piasek et al. 2011).

In summary, blanching and HTD processing are heat processes for the raw berry material, while pasteurization and microwave heating are treatments for the final product. Heat processing of the raw berry materials enhances the release of various phenolic compounds from the berry skin into the berry products, leading to a higher recovery of these compounds, and possible higher retention of the compounds. Additionally, the native pectins remain undisrupted and may protect the phenolic compounds from degradation.

Pasteurization and microwave heating of the final products lead to further degradation of the phenolic compounds. Especially, treatment of juices leads to efficient contact with heat, leading to labile phenolic composition. Pasteurization seems to result in greater degradation of the monomeric anthocyanins, followed by the formation of polymeric color, compared to blanching and HTD processing. In addition, the pectins are usually disrupted in the juices, leading to further lability of the phenolic compounds. All in all, HTD processing and microwave heating were found superior in retention of phenolic compounds compared to pasteurization and blanching.

2.3.5 Jam cooking

During traditional jam cooking, a mixture of berries (at least 45%) and sugar are concentrated by heating to a point at which the content of soluble solids is not less than 65%. Pectins are often added as a jelling agent. Based on the degree of methoxylation, pectins are classified into low methoxy pectins (LM, degree of esterification 25–50%), i.e. calcium gels, and high metoxy pectins

(HM, degree of esterification 50–80%), i.e. acid gels. LM pectins are used in products with low sugar content. LM forms the gel in the presence of Ca^{2+}, which acts as a bridge between the pectin molecules and carboxyl groups. HM pectins are used in high sugar content products in an acid media. HM forms gels with noncovalent forces of hydrogen bonding and hydrophobic interactions. Both types of pectins are relatively stable in jams of low pH (Vibhakara et al. 2012). The temperature and duration of boiling and pasteurization, the recipe, the degree of berry ripeness as well as the storage conditions (temperature, pH, oxygen) of the products are the most important factors determining the quality of berry jams.

Anthocyanins. During jam processing (boiling at 105 °C) a loss of 20–90% in anthocyanin content was detected in boiled raspberries, plums and cherries. The highest loss was observed in plum and the lowest in raspberry jam (Kim & Padilla-Zakour 2004). Lowering the cooking temperature to 70 °C resulted in higher anthocyanin recovery after processing (Holzwarth et al. 2013). The hydrolysis rate of anthocyanins was more affected by the type of sugar attached than the aglycone structure (Howard et al. 2010). As the pH of the jams was low (pH ~3), the rate of anthocyanin hydrolysis seemed to follow the previously established order for acid-catalyzed reactions (Ichiyanagi et al. 2001), where the larger sugars of hexoses (glucoside/galactoside) had greater resistance to hydrolysis than those of the smaller pentoses (arabinoside) (Howard et al. 2010). The high sugar content of jams has been found to stabilize the color due to lower water activities. However, sugar and sugar-free blueberry jams retained the same level of antbocyanins (80%) during jam processing (Howard et al. 2010).

HM and LM pectins were found to stabilize anthocyanins in berry jams more than gelatin (Holzwarth et al. 2013; Maier et al. 2009; Poiana et al. 2012). This was probably due to the formation of hydrocolloidal suspensions of pectins, involving closure of anthocyanin molecules in the pectin gel, creating similar surroundings to encapsulation (Hartmann et al. 2008). The polymeric color values were higher in sugar-free blueberry jam, compared to sugar jam, even though the same level of decrease in anthocyanins occurred in both types of jams (Howard et al. 2010).

Flavonols. Total flavonols and flavonol glycosides remained fairly stable during raspberry and blueberry jam cooking (García-Viguera et al. 1998; Howard et al. 2010; Zafrilla et al. 2001). The stability of flavonols and their glycosides might have derived from the fact that whole berries were used in the jam making without crushing, which would have released oxidizing native enzymes degrading these phenolic compounds. Strawberry jam cooking decreased the content of flavonol aglycones by 15–20% (Häkkinen et al. 2000a).

Tannins and phenolic acids. Blueberry jam cooking resulted in greater losses of the larger oligomers of procyanidins compared to monomers and smaller oligomers (Howard et al. 2010). The greater loss of larger oligomers was most likely due to their binding to cell wall polymers through either a hydrogen bond or hydrophobic interactions, found in the case of apple proanthocyanidins (Le Bourvellec et al. 2004; Renard et al. 2001). Blueberry jams with the addition of sugar also resulted in greater losses of total procyanidins compared to sugar-free jams after processing. Sugar jams with reduced water activity might have enhanced the binding of procyanidins to cell wall polysaccharides or proteins. Sugar jams also had lower levels of procyanidin dimers compared to sugar-free jam. Oligomers of sugar-free jams had possible converted to dimers to higher extent compared to sugar jam. The extraction of dimers might have increased in response to thermal treatment in sugar-free jam (Howard et al. 2010).

During raspberry jam cooking, ellagic acid glycosides remained stable. However, the content of free ellagic acid increased 2.5-fold (Zafrilla et al. 2001). The increase was probably due to the release of hexahydroxydiphenic acid from the ellagitannins and its transformation to ellagic acid. Another reason might be the easier extraction of ellagic acid due to the degradation of cell structures during processing (Odriozola-Serrano et al. 2008b; Zafrilla et al. 2001). The content of chlorogenic acid decreased significantly during jam cooking, but was unaffected by the addition of sugar in blueberry jams (Howard et al. 2010). Food manufacturers are facing challenges to find the optimal conditions for jam making without affecting the level of bioactive compounds too much.

2.3.6 Extrusion cooking

Extrusion cooking is considered to be a high-temperature–short-time processing method capable of preserving desirable food components and destroying micro-organisms. During extrusion, dough-like material goes through homogenization, shearing, thermal cooking, gelatinization and texture alterations, all of which influence the chemical and physical characteristics of the product (Brennan et al. 2011; Maskan & Altan 2012). Extrusion cooking results in end-products with low density and hardness, leading to distinct textural properties, such as crispiness, and crunchiness (Brennan et al. 2011; Meng et al. 2010). The end-products are typically low in moisture, allowing the products to be shelf stable (Brennan et al. 2011; Maskan & Altan 2012).

Extruders consist of either one or two screws inside a horizontal barrel. The dough material is fed into the extruder barrel. The screws transfer the material along the barrel where the food is subjected to increased pressure and shearing, transforming it into a plasticized mass, i.e. melt. At the end of the barrel the

melt is forced through a die (Maskan & Altan 2012). The sudden pressure drop after the die causes bubble nucleation. The pressure difference between the vapor pressure of the water inside the bubbles and the pressure of the melt makes the mass expand to the final shape (Arhaliass et al. 2003). The moisture is flashed off as steam, rapidly cooling the final product (Maskan & Altan 2012).

The technology also improves the bioavailability of the nutrients (Gu et al. 2008), since as the raw materials have undergone so many chemical and structural transformations, which makes them more preferable for the digestive enzymes to release the associated nutrients (Brennan et al. 2011; Saura-Calixto 2010). Extrusion cooking is one viable minimal-processing technology for the utilization of berries, especially the berry pressing side-stream which is still rich in phenolic compounds. The stability of the phenolic compounds is highly dependent on the processing parameters, such as the barrel temperature, screw speed, and moisture content (Brennan et al. 2008).

Anthocyanins. Extrusion cooking resulted in losses of 30–90% of total anthocyanins in cranberry, blueberry and grape press residues, bilberry extract and blueberry and grape concentrate, extruded with various starchy products (Camire et al. 2007; Camire et al. 2002; Chaovanalikit et al. 2003; Hirth et al. 2014; Khanal et al. 2009a; Khanal et al. 2009b; White et al. 2010). The highest loss was observed in the extrudates of blueberry concentrate (Camire et al. 2002).

The process parameters had a great influence on the retention of anthocyanins during extrusion. Greater losses of anthocyanins were obtained with higher barrel temperatures (Hirth et al. 2014; Khanal et al. 2009b; White et al. 2010). Increasing the screw speed retained the anthocyanins to higher extent possibly due to the reduced residence time of the material inside the extruder barrel, minimizing the exposure to high temperatures (Khanal et al. 2009b). However, contradictory results were also obtained, where increased screw speed lowered the retention of anthocyanins in the extruded bilberry extract (Hirth et al. 2014). Barrel temperature and screw speed were found insignificant in retaining the anthocyanins in blueberry and cranberry press residues (Khanal et al. 2009a; White et al. 2010).

Higher feed moisture seemed to prevent anthocyanin degradation in extruded blueberry press residue and bilberry extract (Hirth et al. 2014; Khanal et al. 2009a). The higher moisture probably lowered the shear forces, which cause a rise in temperature inside the barrel. Increasing the flow rate of the dough mixture increased the retention of anthocyanins (Hirth et al. 2014). A high content of starch seemed to protect the anthocyanins in the extrudate mixture (White et al. 2010). Extrusion decreased the polymeric color in blueberry concentrate samples, indicating cleaveges of anthocyanin-

tannin/flavanol polymers. The polymeric color was unaffected in grape sample during extrusion (Camire et al. 2002).

Flavonols. Extrusion cooking increased the content of total flavonols by 30% during extrusion of cranberry press residue (White et al. 2010). One reason for the increased content may be the release of the compounds from skin cell wall components by shear forces during extrusion process.

Tannins. Extrusion enhanced the content of monomers and dimers of procyanidins in cranberry, blueberry and grape press residue and seeds, along with trimers in the case of blueberry. The increase in procyanidin monomers and oligomers with a lower DP could be explained by conversion of oligomers of higher DP values into oligomers with lower DP values. In addition, heat, pressure and shearing effects during extrusion might have facilitated the extraction of these compounds by disruption of the cell wall of the press residues (Khanal et al. 2009a; Khanal et al. 2009b; White et al. 2010). Procyanidin oligomers (DP4–DP9) of the press residues and seeds were reduced during extrusion. Along with the conversion into smaller oligomers, the loss might be explained by the binding of these compounds to cell wall polysaccharides (Khanal et al. 2009b; White et al. 2010). Procyanidins with a lower DP possessed better absorption and thus higher bioactivity in the human intestine system (Shoji et al. 2006). Extrusion could be used as a technology to increase the bioavailability of proanthocyanidins in the berry press residues by converting polymerized proanthocyanidins into molecules with lower DP values.

High barrel temperatures (at 190 °C) seemed to increase the loss of procyanidin oligomers (Khanal et al. 2009b; White et al. 2010), whereas moderately high temperatures (at 170–180 °C) seemed to enhance the contents of monomers and dimers in various press residues (Khanal et al. 2009a; Khanal et al. 2009b; White et al. 2010). Transition seemed to occur at the level of tetramers, which were increased in certain raw berry material under certain extrusion conditions, while being reduced in others (Khanal et al. 2009a; Khanal et al. 2009b; White et al. 2010). Screw speed did not influence the contents of procyanidins in the berry press residues and seeds during the extrusion process (Khanal et al. 2009a; Khanal et al. 2009b; White et al. 2010). The content of certain phenolic acids were found to increase during extrusion, mainly due to release from the cell wall matrix (Brennan et al. 2011).

2.4 Effect of non-thermal processing on phenolic compounds in berry products

Use of supplemental macerating enzymes increases the extraction of juice and phenolic compounds from the berries into the juice. Most commercial enzyme preparations contain endogenous and exogenous enzymes at various ratios, of which some are found also in berries as native enzymes. Technologically, the activities of native enzymes are insufficient, and thus, supplementary macerating enzymes are applied. Pressing berries results in a juice yield of 70–80% as the target product and 20–30% as press residue, consisting of skins, seeds and stems. After the enzymatic extraction of berries, the press residue still contains high amount of anthocyanins, proanthocyanidins and other phenolics, which could be a viable source of natural colorants and bioactive components. Conventional solvent extraction methods often apply heat to enhance the mass transfer, since the phenolic compounds are tightly bound in insoluble structures such as the vacuoles of plant cells. Supplementary enzymes used in berry pressing could also be useful in recovery of the phenolics from the press residue. Non-thermal technologies of pulsed electric field technology and high pressure processes have been beneficial in extracting phenolic compounds from berries and press residues.

2.4.1 Enzyme-assisted extraction

Supplementary enzyme preparations are mixtures of cell wall-degrading pectinases, such as polygalactorunases (EC 3.2.1.15), pectin methylesterases (EC 3.1.1.11) and pectin lyases (EC 4.2.2.2). Additionally, cellulases (EC 3.2.1.4), hemicellulases (EC 3.1.1.73), α-amylases (EC 3.2.1.1), β-amylases (EC 3.2.1.2), and cutinases (EC 3.1.1.74) are used in various ratios together with pectin degrading enzymes (Buchert et al. 2005; Kashyap et al. 2001; Koponen et al. 2008b; Landbo & Meyer 2001; Nyyssölä 2015; Versari et al. 1997). The release of phenolic compounds into juice is highly dependent on the ability of the enzymes to degrade berry cell wall polysaccharides during juice processing. The down-side of supplemental enzyme preparations is the possible glycosidase side-activities, such as α-arabinosidase, β-galactosidase and β-glucosidase. These activities may hydrolyze the glycosyl moiety of phenolic compounds, resulting in corresponding aglycones (Buchert et al. 2005; Jiang et al. 1990; Kapasakalidis et al. 2009; Koponen et al. 2008a; Koponen et al. 2008b; Puupponen-Pimiä et al. 2008; Wightman & Wrolstad 1995). The impact of the activities of enzyme preparations on the phenolic compounds of blueberries/bilberries, black currants, raspberries, and grapes are summarized in Table 1. The studies summarize the use of berries or press residues as raw

material in enzymatic pressing of berries or recovery of phenolic compounds from press residues.

Anthocyanins. Enzyme preparations with high polygalacturonase, pectin methylesterase and pectin lyase activities resulted in the highest content of anthocyanins in the various berry juices (Table 1) (Bagger-Jørgensen & Meyer 2004; Dal Magro et al. 2016; Heffels et al. 2016; Laaksonen et al. 2012; Landbo & Meyer 2001; Landbo & Meyer 2004; Puupponen-Pimiä et al. 2008). Berries with a thicker skin and higher content of pectin, such as black currants, need higher dosages of pectinolytic enzymes for proper anthocyanin yields, compared to berries with a soft skin and lower pectin content, such as bilberries (Buchert et al. 2005; Koponen et al. 2008a; Landbo & Meyer 2004). Hemicellulase, cellulase and protease activitives were found particularly useful in releasing anthocyanins from grapes and grape press residues into the juice and extracts (Table 1) (Costoya et al. 2010; Dal Magro et al. 2016; Tomaz et al. 2016). Proteases might be useful in hydrolysing the structural proteins around the primary cell wall and vacuoles for a more thorough release of phenolic compounds (Costoya et al. 2010).

Treatment conditions for enzyme maceration have a great impact on anthocyanin extraction into berry juices and their release from press residues. Increased doses of supplementary enzymes, maceration time, and reaction temperature increase the anthocyanin content in the juices. The same increase in the parameters were found to elevate the recovery of anthocyanins from press residues (Gavrilas & Stanescu 2016; Laaksonen et al. 2012; Landbo & Meyer 2004; Landbo et al. 2007; Laroze et al. 2010; Toaldo et al. 2014). The side-activities of enzyme preparations resulted in unstable aglycones and color loss in the berry juices (Buchert et al. 2005; Jiang et al. 1990; Kapasakalidis et al. 2009; Koponen et al. 2008a; Koponen et al. 2008b; Puupponen-Pimiä et al. 2008; Wightman & Wrolstad 1995).

The recommended enzyme dosages increased the content of anthocyanins, without affecting the profile by side-activities (Heffels et al. 2016). Anthocyanin destruction by the glycosidase side-activity often indicated higher than recommended enzyme dosages (Heffels et al. 2016; Versari et al. 1997). The profile of the four main anthocyanins did not change during the enzyme-aided processing of black currants in comparison to berries (Iversen 1999), even though the yields of anthocyanins almost doubled (Landbo & Meyer 2004).

Cell wall degrading enzymes seemed to have a synergistic effect on anthocyanin extraction. When processing grape skin, anthocyanin levels were significantly higher with enzyme mixtures of pectinases, hemicellulases, and cellulases, than with only one of these enzymes, (Costoya et al. 2010; Toaldo et al. 2014; Tomaz et al. 2016). Cutinases have been useful in accelerating drying

of grapes by enhancing the water permeability of the tissues. There are a few patents concerning treatments with cutinases on fruits and vegetables, but no studies were found about their usage on berries.

Flavonols. Enzymatic treatment of berries and press residues significantly increased the yields of flavonol glycosides in the juice and their recovery from press residue (Dal Magro et al. 2016; Koponen et al. 2008b; Tomaz et al. 2016). Glycosidase side-activities of the enzyme preparations may degrade the flavonol glycosides into corresponding aglycones in the berry juice (Table 1) (Bagger-Jørgensen & Meyer 2004; Buchert et al. 2005; Dal Magro et al. 2016; Koponen et al. 2008a; Koponen et al. 2008b; Laaksonen et al. 2012; Wilkes et al. 2014), especially at a longer maceration time (Tomaz et al. 2016).

Regarding red grapes, enzyme preparation with the highest cellulase activity resulted in the highest yields of flavonol glycosides in the juice, compared to the activities of polygalacturonase, pectin lyase and pectin methylesterase (Dal Magro et al. 2016). For flavonol extraction, the optimum dosages of enzymes were lower than the optimum dosages of enzymes for anthocyanin extraction in black currants and bilberries (Koponen et al. 2008b). During pectinase treatment, the extractability of individual flavonol glycosides varied, resulting in changes in the flavonol profiles of juices compared to unprocessed berries. The relative extractablity of rutinosides was higher than that of glucosides among flavonol glycosides (Koponen et al. 2008b). This indicates that the flavonol glycosides either have varying affinities to the berry cell wall matrixes, or they have differences in solubility.

Tannins. Black currants were macerated with pectinases with a dosage of 15 mg/100 g of berry mash at 45 °C for 4 hours. Pectinase treatment increased the content of proanthocyanidins in the juice 5–14-fold, compared to non-enzymatic pressing. However, the increase was highly dependent on the variety. The majority of the proanthocyanidins in black currant juices had DP higher than three. The contents of these compounds increased 7–20-fold by enzyme treatment (Laaksonen et al. 2015). Cranberry and chokeberry proanthocyanidins could only just be extacted with pectinases at dosages of approximately 0.12% w/v and 10 mg/100g of berry mash, respectively, and with maceration at 45 °C for 1 hour (White et al. 2011; Wilkes et al. 2014). Higher pectinase dosages and a higher maceration time would be beneficial in extracting the proanthocyanidins in cranberries and chokeberries. Commercial pectinases decreased the content of ellagic acid derivatives in raspberry juice, whereas the content of these compounds increased in strawberry juices, even increasing over the time of the treatment (Versari et al. 1997).

Phenolic acids. Enzyme preparation with high hemicellulase-cellulase activity released high amounts of phenolic acids in the juice and increased the recovery of the phenolic acids from press residues of red grapes and black

currants. Hemicellulase-cellulase activity seemed to be more efficient in extracting phenolic acids from berry material compared to activities of polygalacturonase, pectin lyase and pectin methylesterase (Dal Magro et al. 2016; Kapasakalidis et al. 2009; Laaksonen et al. 2012). In addition, pectinases increased the extraction of *p*-coumaric acid derivatives in black currants. The release was higher with a higher enzyme dosage (Laaksonen et al. 2012). Hydroxycinnamic acids required a longer maceration time compared to hydroxybenzoic acids. Higher macerating temperature increased the extractability of hydroxybenzoic acids (Kapasakalidis et al. 2009). A proper enzyme preparation and dosage need to be chosen for every berry species according to their pectin content and phenolic composition in order to maximize the juice and phenolic content in the juices and at the same time, minimize the decomposition of phenolic compounds by the enzymes.

2.4.2 Pulsed electric field processing

Pulsed electric field (PEF) processing is a non-thermal technology that was approved in the USA in 1995 for antimicrobial treatment of liquid foods (Morris 2000). In PEF technology, an electric field strength of 1–20 kV/cm or high-intensity field strength of 20–80 kV/cm is applied to food, in the form of short- or high-voltage pulses (µs–ms), placed between two metal electrodes (**Figure 6**) (Buckow et al. 2013; Lamanauskas et al. 2016; Odriozola-Serrano et al. 2008a). PEF is suitable mainly for liquid raw materials, since liquid foods contain ions that function as electrical conductors (Zhang et al. 1995). An external electric field induces a critical electric potential across the cell membrane of the food system inducing electroporation (Uchida et al. 2008; Zhao et al. 2010). Electroporation increases cell permeability, which leads to cellular damage and accelerates the mass transport (Leong et al. 2016). These effects result in inactivation of microbes (Elez-Martínez et al. 2004; Elez-Martínez et al. 2006; Rodrigo et al. 2001) and native enzymes (Elez-Martínez et al. 2006; Giner et al. 2005; Marsellés-Fontanet & Martin-Belloso 2007). At the same time, the increased permeability of the berry cell membrane improves juice flow from the berries (Grimi et al. 2009; Leong et al. 2016; Praporscic et al. 2007), and extraction of phenolic compounds from the skin into the juice (Lamanauskas et al. 2016; Leong et al. 2016). An electric field strength of 35 kV/cm with pulses of 5000 µs was sufficient to inactive PPO in white grape juice (Marsellés-Fontanet & Martin-Belloso 2007). POD was inactivated at 35 kV/cm with pulses of 4 µs in orange juice (Elez-Martínez et al. 2006).

Anthocyanins. PEF technology has been developed as an alternative to thermal pasteurization, resulting in e.g. juices with higher amounts of health-related phenolic compounds and aromas and lower losses of color, flavor and

taste (Odriozola-Serrano et al. 2013). PEF processing (35 kV/cm with pulses of 4 µs) retained the content of anthocyanins in strawberry juices to a higher extent compared to pasteurization (at 90°C for 60 s) (Odriozola-Serrano et al. 2008b). In strawberry juice treated with high-intensity pulsed electric fields (20–35 kV/cm with pulses of 1 µs), higher electric field strength and lower treatment time led to higher retention of anthocyanins (Odriozola-Serrano et al. 2008a).

PEF was found to be a viable pre-treatment method in juice pressing. PEF was applied as a pre-treatment for blueberries and grapes (0.4–5 kV/cm with pulses of 20–1000 µs) immediately before juice extraction. The content of anthocyanins increased to a higher level in the juices pressed with PEF treated berries, compared to juices pressed with untreated berries (Bobinaitė et al. 2015; Grimi et al. 2009; Lamanauskas et al. 2015; Leong et al. 2016). In the PEF pre-treated blueberries, the anthocyanin yield did not further increase in the juice with higher field strength than 1 kV/cm (with pulses of 20 µs) statistically significantly, but rather decreased (Bobinaitė et al. 2015). In another study, only a field strength of 3 kV/cm (with pulses of 20 µs) resulted in a slight increase in the content of anthocyanins in blueberry juice pressed with PEF pre-treated berries (Lamanauskas et al. 2015). Pre-treatment on raspberries (1 or 3 kV/cm with pulses of 20 µs) did not have a significant impact on the anthocyanin level of the juice (Lamanauskas et al. 2016).

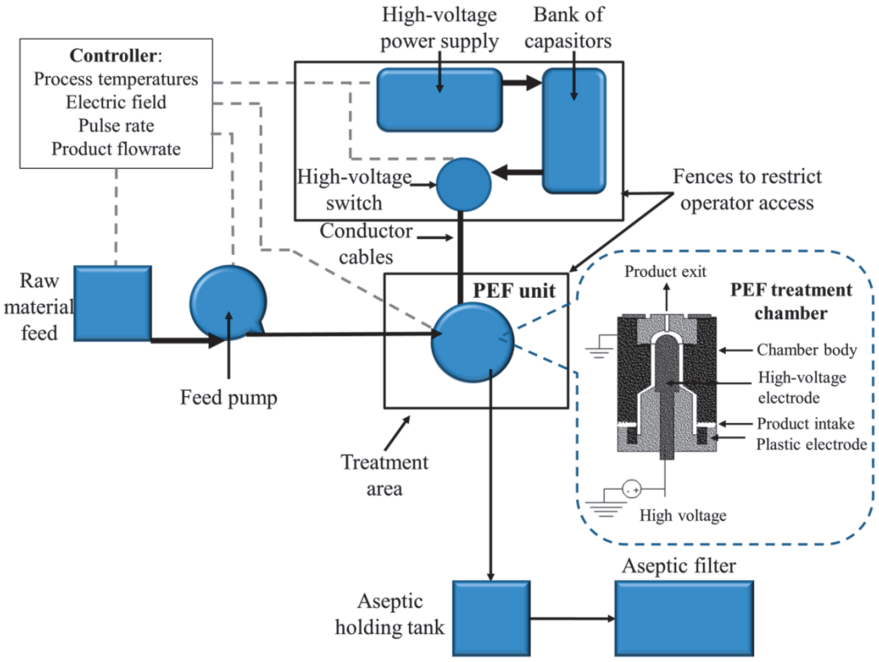

Figure 6. Schematic diagram of PEF processing equipment. Adapted from Fellows 2009 and reproduced with permission. Copyright 2009 Elsevier.

Table 1 Comparison of commercial macerating enzymes in extraction of phenolic compounds from berries and press residues of blueberries/bilberries, black currants, raspberries and grapes.

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
Biopectinase CCM	Polygalacturonase (EC 3.2.1.15), pectin methylesterase (EC 3.1.1.11), mannanase (EC 3.2.1.78), xylanase (EC 3.2.1.8), α-arabinosidase (EC 3.2.1.55), endoglucanase (EC 3.2.1.4), β-galactosidase (EC 3.2.1.23) and β-glucosidase (EC 3.2.1.21)	Bilberry and black currant	Bilberry: Improved anthocyanin extractability by 10–20%. Higher hydrolysis of flavonol conjugates compared to Econase CE and Pectinex Smash XXL, resulting in decreased contents of flavonol galactosides and glucuronides. Increased the levels of aglycones. Black currant: Increased the content of anthocyanins by 30% in the juice.	Buchert et al. 2005; Koponen et al. 2008a; Koponen et al., 2008b; Puupponen-Pimiä et al. 2008
Biopectinase Super 8X	Polygalacturonase, pectin methylesterase, α-arabinosidase, β-galactosidase, pectin lyase (EC 4.2.2.10) and β-glucosidase	Black currant and bilberry	Black currant:- Bilberry: Increased the content of phenolic compounds and anthocyanins.	Laaksonen et al. 2012; Puupponen-Pimiä et al. 2008
Cellubrix	Cellulase (EC 3.2.1.4), hemicellulose (EC 3.1.1.73) and β-glucosidase	Raspberry press residue; red and white grape press residue	Raspberry press residue: Decreased the content of total phenolic compounds. Red and white grape press residue : -	Costoya et al. 2010; Laroze et al. 2010
Cellulase 13P	β-Glucanase and endoglucanase	Black currant	-	Laaksonen et al. 2012
Depol 740L	β-Glucanase and endoglucanase	Black currant	-	Laaksonen et al. 2012
E1	Experimental stages, no information provided by the manufacturer	Blueberries and blueberry skins	Produced the least amount of polymeric color during the treatment of the berry and the skin compared to E2–E9.	Lee & Wrolstad 2004
E2	Pectinase and hemicellulase	Blueberries and blueberry skins	-	Lee & Wrolstad 2004
E3	Cellulase	Blueberries and blueberry skins	-	Lee & Wrolstad 2004

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
E4	Pectinase, cellulase and cellobiase	Blueberries and blueberry skins	-	Lee & Wrolstad 2004
E5	Pectinase	Blueberries and blueberry skins	-	Lee & Wrolstad 2004
E6	Experimental pectinase, cellulase and cellobiase	Blueberries and blueberry skins	Lower extraction of total phenolics and anthocyanins from berries compared to E1–E5; E7–E9.	Lee & Wrolstad 2004
E7	Pectinase and cellulase	Blueberries and blueberry skins	Higher levels of anthocyanins and polymeric color from the treatment of skin compared to E1–E6; E8, E9.	Lee & Wrolstad 2004
E8	Pectinase and hemicellulase	Blueberries and blueberry skins	Higher extraction of anthocyanins from the treatment of berries compared to E1–E7, E9.	Lee & Wrolstad 2004
E9	Pectinase	Blueberries and blueberry skins	-	Lee & Wrolstad 2004
Econase CE	Xylanase, endoglucanase, mannanase, polygalacturonase, α-arabinosidase, β-galactosidase and β-glucosidase	Bilberry and black currant	Bilberry: Decreased the content of anthocyanins by 90% due to high α-arabinosidase activity. Hydrolyzed anthocyanidin glucosides. Black currant: Improved the extractability of phenolics by 15%	Buchert et al. 2005; Koponen et al. 2008a; Koponen et al. 2008b
Endozym Contact Pelliculaire	Polygalacturonase, pectin lyase, pectin methylesterase, cellulase and hemicellulase	Red grape skin	-	Tomaz et al. 2016
Endozym Rouge	Pectin lyase, polygalacturonase, pectin methylesterase, cellulase and hemicellulase	Red grape skin	-	Tomaz et al. 2016
Enovin Color	Polygalacturonase, β-glucanase,	Raspberry	Higher yields of polyhydroxyphenolics compared to Enozym Vintage	Gavrilas & Stanescu 2016

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
	pectin lyase and pectin methylesterase			
Enozym Vintage	Polygalacturonase, β-glucanase, pectin methylesterase, pectin lyase	Raspberry	Lower yields of polyhydroxyphenolics compared to Enovin Color.	Gavrilas & Stanescu 2016
Enozym Altair	Polygalacturonase, pectin methylesterase and pectin lyase	Raspberry	-	Gavrilas & Stanescu 2016
Everzym® Color	Polygalacturonase and hemicellulase	Red grape	Higher concentrations of monomeric anthocyanins compared to Rapidase Smart.	Toaldo et al. 2014
Granozyme	Xylanase	Raspberry press residue	Decreased the content of total phenolic compounds	Laroze et al. 2010
Grindamyl CA 150	Pectinase	Raspberry press residue	Higher recovery of polyphenols, compared to Cellubrix, Olivex, Pectinex Ultra SP-L, Ultrazym 100G, Viscozym, Rohapect 10L, Rohapect DA6L, Rohapect Max, Rohavin L and Granozyme	Laroze et al. 2010
Grindamyl pectinase	Pectinase, cellulase and hemicellulase	Black currant; black currant press residue	-	Bagger-Jørgensen & Meyer 2004; Landbo & Meyer 2001
Grindamyl Pectinase LM	Not given	Raspberry	Decreased the content of ellagic acid derivatives.	Versari et al. 1997
Grindamyl Pectinase LX	Not given	Raspberry	Decreased the content of ellagic acid derivatives.	Versari et al. 1997
Klerzyme Color	Pectinase and protease	Black currant	Increased the content of anthocyanins.	Landbo & Meyer 2004
Lallzyme Beta	Polygalacturonase, cellulase, and pectin lyase	Red grape	Higher increase (60%) in anthocyanins and higher content of total phenolic acids, compared Pectinex Ultra SP-L, Pectinex Ultra Color, Pectinex Smash XXL, Novozym 33095, Pectinex Ultra Clear, Pectinex BE XXL and Rohapect 10 L. Provided high concentration of quercetin glucoside and increased the content of quercetin aglycone by 50%.	Dal Magro et al. 2016
Lallzyme EX-V	Polygalacturonase, pectin methylesterase,	Red grape skin	Increased the extraction of anthocyanins, flavonol glycosides and flavan-3-ols	Tomaz et al. 2016

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
	pectin lyase, cellulase and hemicellulase			
Lallzyme HC	Polygalacturonase, pectin methylesterase and pectin lyase	Red grape skin	More efficient in extracting flavonoids compared to Endozym Contact Pelliculaire and Endozym Rouge.	Tomaz et al. 2016
Laminex®	Cellulase	Red grape press residue and grape seed extract	Reduced the content of epicatechin *O*-gallate by 70% in the press residue.	Chamorro et al. 2012)
Macer8™W	β-Glucanase, polygalacturonase, endoglucanase, α-arabinosidase, β-galactosidase, β-glucosidase, pectin methylesterase and pectin lyase	Black currant	Higher contents of individual anthocyanins and flavonol aglycones, compared to Biopectinase Super 8X, Rapidase BE Super and Grindamyl pectinase. Higher increase in the content of flavonol aglycones and hydroxycinnamic acid derivatives compared to Cellulase 13P, Depol 740L, Biopectinase Super 8X and Pectinex 5XL.	Bagger-Jørgensen & Meyer 2004; Laaksonen et al. 2012
Macer8™ FJ	Pectinase	Black currant; black currant press residue	Decreased the recovery of anthocyanins in black currant press residue probably due to glycosidase activities.	Landbo & Meyer 2001; Landbo & Meyer 2004
Macer8 R	Pectinase	Black currant press residue	Decreased the recovery of anthocyanins in black currant press residue probably due to glycosidase activities.	Landbo & Meyer 2001
Maxoliva	Pectinase	Raspberry press residue	Higher recovery of polyphenols, compared to Cellubrix, Olivex, Pectinex Ultra SP-L, Ultrazym 100G, Viscozym, Rohapect 10L, Rohapect DA6L, Rohapect Max, Rohavin L and Granozyme	Laroze et al. 2010
Neutrase	Protease and α-amylase	Red and white grape press residue	Higher release of phenolic compounds, compared to Cellubrix and Viscozyme	Costoya et al. 2010
Novozym 33095	Polygalacturonase, pectin methylesterase, pectin lyase and cellulase	Red grape	Provided high concentration of quercetin glucoside and increased the content of quercetin aglycone by 50%.	Dal Magro et al. 2016
Novozym 89	Not given	Black currant	No effect on anthocyanin yield.	Landbo & Meyer

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
acid protease		press residue		2001
Olivex	Pectinase and hemicellulase	Raspberry press residue	-	Laroze et al. 2010
Panzym BE XXL	Pectinase	Bilberry skin	Increased the extraction of anthocyanins.	Dinkova et al. 2014
Panzym Pro Color	Pectin lyase	Bilberry skin	Increased the extraction of anthocyanins.	Dinkova et al. 2014
Pectinex 3 XL	Polygalacturonase, α-arabinosidase, pectin methylesterase, β-galactosidase, xylanase, endoglucanase, mannase and β-glucosidase	Bilberry; raspberry	Bilberry:- Raspberry: Lower extraction of cyanidin sophoroside, compared to Pectinex BE 3L, Rohapect MB, Rohapect B1L, Rohament MAX, Grindamyl Pectinase LX and Grindamyl Pectinase LM.	Puupponen-Pimiä et al. 2008; Versari et al. 1997
Pectinex 5XL	β-glucanase, polygalacturonase, pectin methylesterase, endoglucanase, β-glucosidase and pectin lyase	Black currant	-	Laaksonen et al. 2012
Pectinex BE	Pectin methylesterase, pectin lyase and polygalaturonase	Black currant; black currant press residue	Black currant: Higher increase of anthocyanin yields in the juice, compared to Macer8^{TM}, Klerzyme Color, Pectinex Superpress, Pectinex Ultra SP-L, Rapidase BE Super, Rapidase EX Color, Rapidase Vino Super, Rohapect B5L and Vinozyme G. Press residue: no effect on anthocyanin yield.	Landbo & Meyer 2001; Landbo & Meyer 2004
Pectinex BE-3L	Xylanase, polygalacturonase, α-arabinosidase, β-galactosidase, pectin methylesterase, mannanase,	Bilberry and black currant; bilberry; raspberry	Bilberry: Higher increase (40%) in anthocyanin content, compared to Pectinex Ultra SP-L, Pectinex Smash and Biopectinase CCM. High α-arabinosidase activity hydrolyzed anthocyanidin arabinosides by 30%. Lower levels of anthocyanins compared to Pectinex Ultra SP-L, Pectinex Smash XXL, Pectinex 3 XL,	Buchert et al. 2005; Koponen et al. 2008a; Koponen et al., 2008b; Puupponen-Pimiä et al. 2008; Versari et al. 1997

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
	endoglucanase and β-glucosidase		Pectinex Smash, Pectinex BE XXL, Rohapect, Biopectinase and Biopectinase Super 8X. Higher hydrolysis of flavonol conjugates compared to Econase CE and Pectinex Smash XXL, resulting in increased levels of aglycones. Black currant: Improved the extractability of phenolic compounds by 50%. Raspberry:-	
Pectinex BE Colour	Polygalacturonase, pectin methylesterase and pectin lyase	Black currant and plum	-	Mieszczakowska-Frąc et al. 2012
Pectinex BE XXL	Polygalacturonase, pectin methylesterase, pectin lyase and cellulase	Bilberry; red grape; black currant and plum	Bilberry: Higher increase (40%) in anthocyanin content, compared to Rohapect PTE 100, Rohament CL, Vinozym Ultra FCE, Rohapect 10 L and Vegazym HC. Red grape: Higher increase of anthocyanins (40%) compared to Pectinex Ultra SP-L, Pectinex Ultra Color, Pectinex Smash XXL, Novozym 33095 and Pectinex Ultra Clear. Black currant and plum: -	Dal Magro et al. 2016; Heffels et al. 2016; Mieszczakowska-Frąc et al. 2012; Puupponen-Pimiä et al. 2008
Pectinex Smash	Polygalacturonase, mannanase, pectin methylesterase, endoglucanase, β-galactosidase, α-arabinosidase, xylanase and β-glucosidase	Bilberry and black currant	Bilberry: Improved the extractability of anthocyanin by 10–20%. Black currant: -	Buchert et al. 2005; Puupponen-Pimiä et al. 2008
Pectinex Smash XXL	Pectin lyase and cellulase	Red grape; bilberry and black currant	Red grape: - Bilberry and black currant: Higher anthocyanin yields compared to Econase CE, Biopectinase CCM and Pectinex BE 3-L. Bilberry: Higher dosages led to losses of flavonols.	Dal Magro et al. 2016; Koponen et al. 2008a; Koponen et al. 2008b; Puupponen-Pimiä et al. 2008

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
Pectinex Superpress	Pectin transeliminase (EC 4.2.2.2), polygalacturonase, pectin methylesterase and hemicellulase	Black currant	Increased the content of anthocyanins.	Landbo & Meyer 2004
Pectinex Ultra Clear	Polygalacturonase, pectin methylesterase, pectin lyase and cellulase	Red grape	-	Dal Magro et al. 2016
Pectinex Ultra Color	Polygalacturonase, pectin methylesterase, pectin lyase and cellulase	Red grape; bilberry skin	Red grape: -. Bilberry skin: Higher extractability of anthocyanins compared to Panzym Pro Color and Panzym BE XXL.	Dal Magro et al. 2016; Dinkova et al. 2014
Pectinex Ultra SP-L	Polygalacturonase, mannanase, pectin methylesterase, endoglucanase, β-galactosidase, xylanase, α-arabinosidase and β-glucosidase	Bilberry and black currant; red grape; raspberry press residue.	Bilberry: Improved extractability of anthocyanins by 10–20%. Higher increase in total phenolic compounds compared to treatments with Rapidase BE Super and Grindamyl pectinase. Black currant: Increased the content of anthocyanins by 30% in the juice. Higher increase in total phenolic compounds compared to treatments with Rapidase BE Super and Grindamyl pectinase. Red grapes:- Raspberry press residue:-	Bagger-Jørgensen & Meyer 2004; Buchert et al. 2005; Dal Magro et al. 2016; Landbo & Meyer 2004; Laroze et al. 2010; Puupponen-Pimiä et al. 2008
Pektozyme®	Pectin lyase	Red grape press residue and grape seed extract	Increased the content of gallic acid by 30% and epigallocatechin by 20%, and reduced the content of epicatechin *O*-gallate in the press residue.	Chamorro et al. 2012
Rapidase BE Super	Pectinase and hemicellulase	Black currant	Increased the content of anthocyanins.	Bagger-Jørgensen & Meyer 2004; Landbo & Meyer 2004
Rapidase EX Color	Pectinase	Black currant	Minor increase in the anthocyanin content.	Landbo & Meyer 2004
Rapidase®	Polygalacturonase and	Red grape	Lower concentrations of total phenolics and monomeric	Toaldo et al. 2014

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
Smart	pectin methylesterase		anthocyanins compared to Everzym Color.	
Rapidase Vino Super	Not given	Black currant	Increased the content of anthocyanins.	Landbo & Meyer 2004
Rohament CL	Polygalacturonase and *β*-glucosidase	Bilberry	Increased anthocyanin content by 10%	Heffels et al. 2016
Rohament MAX	Not given	Raspberry	-	Versari et al. 1997
Rohament PL	Polygalacturonase, pectin methylesterase and pectin lyase	Black currant and plum	-	Mieszczakowska-Frąc et al. 2012
Rohapect	Xylanase, polygalacturonase, mannase, endoglucanase, β-galactosidase, β-glucosidase and α-arabinosidase	Bilberry	-	Puupponen-Pimiä et al. 2008
Rohapect 10 L	Polygalacturonase, pectin methylesterase, pectin lyase and cellulase	Bilberry; red grape; raspberry press residue	Bilberry:- Red grape: Higher increase in anthocyanin (by 50%) compared to Pectinex Ultra SP-L, Pectinex Ultra Color, Pectinex Smash XXL, Novozym 33095 and Pectinex Ultra Clear. Raspberry press residue: Decreased the content of total phenolic compounds	Dal Magro et al. 2016; Heffels et al. 2016; Laroze et al. 2010
Rohapect B1L	Not given	Raspberry	-	Versari et al. 1997
Rohapect B5L	Pectinase	Black currant	Increased the content of anthocyanins to a greater extent, compared to Macer8, Pectinex Superpress, Pectinex BE, Pectinex Ultra SP-L, Rapidase BE Super, Rapidase EX Color, Klerzyme Color, Rapidase Vino Super and Vinozyme G.	Landbo & Meyer 2004
Rohapect DA6L	Pectinase	Raspberry press residue	Degraded the content of total phenolic compounds	Laroze et al. 2010
Rohapect Max	Pectinase	Raspberry press residue	Increased the extraction of phenolic compounds	Laroze et al. 2010

Enzyme product	*Activity profile* [a]	*Berry essayed*	*Impact of the enzyme on phenolic compounds*	*Ref.*
Rohapect MB	Not given	Raspberry	Higher increase in quercetin derivatives compared to Pectinex BE 3L, Pectinex 3XL, Rohapect B1L, Rohament MAX, Grindamyl Pectinase LX and Grindamyl Pectinase LM.	Versari et al. 1997
Rohapect PTE	Pectin lyase, polygalacturonase and pectin methylesterase	Black currant and plum	-	Mieszczakowska-Frąc et al. 2012
Rohapect PTE 100	Polygalacturonase and *β*-glucosidase	Bilberry	Lower anthocyanin yields compared to Rohament CL, Pectinex BE XXL, Vinozym Ultra FCE, Rohapect 10 L and Vegazym HC.	Heffels et al. 2016
Rohavin L	Pectinase and cellulase	Raspberry press residue	-	Laroze et al. 2010
Tannase	Tannin acyl hydrolase (EC 3.1.1.20)	Red grape press residue and grape seed extract	Increased the content of gallic acid and reduced the content various gallic acid derivatives.	Chamorro et al. 2012
Ultrazym 100G	Pectinase	Raspberry press residue	Decreased the content of total phenolic compounds	Laroze et al. 2010
Vegazym HC	Polygalacturonase and *β*-glucosidase	Bilberry	Minor increase in the anthocyanin content when higher enzyme dosage was used	Heffels et al. 2016
Vinozym Ultra FCE	Polygalacturonase and *β*-glucosidase	Bilberry	Increased the content of anthocyanins.	Heffels et al. 2016
Vinozyme G	Pectin lyase, polygalacturonase, hemicellulase and cellulase	Black currant	Decreased the content of anthocyanins.	Landbo & Meyer 2004
Viscozym	Arabanase, cellulase, β-glucanase, hemicellulase and xylanase	Raspberry press residue; red and white grape press residue	Raspberry press residue: Decreased the content of total phenolic compounds. Red and white grape press residue: -	Costoya et al. 2010; Laroze et al. 2010

[a] Enzyme activies (EC) are numbered after IUBMB Enzyme Nomenclature (IUBMB Enzyme Nomenclature). The activities are placed in order by decreasing activity of the enzyme preparate. (-) No special info about the impact of the enzyme on phenolic compound.

PEF technology was compared to ultrasonication on berry purées. PEF treatment (not mentioned as kV/cm) of blueberry purée applied alone and in combination with ultrasonication (400 W at 24 kHz) increased the extraction of anthocyanins, compared to ultrasonication treatment applied alone. Anthocyanin extraction increased to a higher level with the combination of PEF and ultrasonication. In the case of raspberry purées, the extraction of anthocyanins increased only when PEF was applied alone (Medina-Meza et al. 2016). The varying impact of the treatment might be derived from the different distributions of anthocyanins in the berry structures (Lee & Wrolstad 2004; Monagas et al. 2006; Pinelo et al. 2006; Sablani et al. 2010). In the case of grape and plum peels, the mechanical action of ultrasonication together with heat (50 °C) was found to be a better treatment for efficient extraction of skin anthocyanins compared to PEF (Medina-Meza & Barbosa-Cánovas 2015).

Ultrasonication applied alone had either no impact or a deleterious effect on the anthocyanin extraction of blueberry or raspberry purées (Medina-Meza et al. 2016). The deleterious effect of ultrasound on anthocyanin yield may be explained by the harsher treatment of cavitation, compared to PEF treatment. Cavitation generates a high local temperature, pressure and mechanical action producing ruptures and holes on cell wall membranes (Fava et al. 2006). These actions may liberate anthocyanins from broken vacuoles, which then come in contact with oxidizing enzymes of PPO and POD (Cheynier & da Silva 1991). Another explanation could be the cavitational thermolysis, which may generate free radicals degrading anthocyanin structures into chalcones. Cavitational thermolysis may also enhance polymerization reactions (Floros & Liang 1994; Portenlänger & Heusinger 1997; Vercet et al. 1998). The increase in anthocyanins in response to PEF treatment indicates that PEF treatment results in more gentle and selective damage to the membranes, compared to ultrasonication (Fincan & Dejmek 2002).

PEF treatment was found useful in anthocyanin extraction from berry press residues. The anthocyanin yield increased along with increasing field strength up to 110% in blueberry press residue, compared to untreated residue (Bobinaitė et al. 2015). In raspberry press residues, the anthocyanin yield did not increase statistically significantly after 1 kV/cm of field strength (with pulses of 20 μs), but rather decreased (Lamanauskas et al. 2016). PEF treatment (3 kV/cm, with a series of 30 pulses to obtain 10 kJ/kg) increased the extraction of anthocyanin monoglucosides to a higher extent compared to acylated glucosides of grape by-products. Acylated glucosides seemed to be physically entrapped within the matrix, or they formed hydrogen bonds with cell wall polysaccharides and were consequently extracted in lower proportions (Corrales et al. 2008). The use of stainless steel as the electrode material was a

better choice than pure titanium and titanium based alloy in retaining the highest content of cyanidin glucoside in the model solutions (Sun et al. 2011).

Flavonols. PEF treatment (not mentioned as kV/cm) of blueberry purée increased the level of flavonols. However, PEF had no impact on flavonols in raspberry purée. Ultrasonication (400 W at 24 kHz) alone or in combination with PEF increased the flavonol content of both of the berry purées to a higher level when compared with the application of PEF alone (Medina-Meza et al. 2016). PEF and ultrasonication treatments applied on plum peel enhanced the extraction of flavonols from the peels (Medina-Meza & Barbosa-Cánovas 2015). Neither PEF processing (35 kV/cm with pulses of 4 μs) nor pasteurization (at 90 °C for 60 s) had an effect on the content of flavonols (kaempferol, quercetin and myricetin) in strawberry juices (Odriozola-Serrano et al. 2008b).

Tannins and phenolic acids. In strawberry juices, pasteurization (at 90°C for 60 s) reduced the content of ellagic acid, whereas the compound remained stable with PEF processing (35 kV/cm with pulses of 4μs) (Odriozola-Serrano et al. 2008b). No statistical differences were observed in the contents of cinnamic acid and free catechin between red gape juices treated with PEF (35 kV/cm with pulses of 4 μs) and thermal treatment (at 90 °C for 1 min) (Marsellés-Fontanet et al. 2013).

2.4.3 High pressure processing

High hydrostatic pressure (HHP) technology is an alternative preservation method to conventional thermal treatments. HHP treatment reduces the effects on phenolic compounds and other nutritional and quality parameters compared to heat treatment (Oey et al. 2008a; Rastogi et al. 2007; Rawson et al. 2011). High-pressure pasteurization has been extensively studied and already applied in industrial processes (Oey et al. 2008a). Packed or unpacked foods are subjected to HHP in water at pressures of 100–900 MPa for 1–20 min at an ambient temperature. The high pressure mainly affects the noncovalent bonds, which is detrimental to the micro-organisms, but preserves all the micronutrients, pigments, and flavor compounds well (Hendrickx et al. 1998; Oey et al. 2008a). High pressure causes plant cell disruption by disrupting salt bridges and hydrophobic bonds, thus leading to a higher extractability of phenolic compounds from berry juices and purées (Jiménez-Sánchez et al. 2015; Patras et al. 2009; Rastogi et al. 2003). HHP treatment at 600 MPa for 15–25 min at room temperature is able to inactivate native oxidizing enzymes partially or substantially, or in some cases even completely, in berries and berry products (Cao et al. 2011; Cao et al. 2012; Garcia-Palazon et al. 2004).

Anthocyanins. Anthocyanins in various berry products were reported to be stable, or even increase, during high-pressure treatment up to 600 MPa for 5–25 min at room temperature (Barba et al. 2013; Cao et al. 2011; Gimenez et al. 2001; Kouniaki et al. 2004; Patras et al. 2009; Tadapaneni et al. 2014). At these treatment parameters, the high pressure seemed to have an impact only on molecules involved in the kinetics of reaction, such as enzymes. Additionally, the relation between monomeric and polymeric anthocyanins was not modified significantly in comparison to fresh blueberry juices (Barba et al. 2013). Elevated pressures and temperatures (up to 700 MPa at 95–130 °C) resulted in degradation of anthocyanins in various berry products, the impact of temperature being obviously more significant in comparison to HHP (Gimenez et al. 2001; Tadapaneni et al. 2012; Verbeyst et al. 2010; Verbeyst et al. 2011). However, at pressures higher than 700 MPa, the reduction of anthocyanins did not differ from heat treatments in strawberry beverages (Tadapaneni et al. 2012). The stability of strawberry and red raspberry anthocyanins at 800 MPa for 15 min might have been due to a complete inactivation of PPO (Garcia-Palazon et al. 2004). The best stability of anthocyanins was reached at a pressure of 200 MPa in strawberry jams (Gimenez et al. 2001). The lower water activity of jams compensated for the possible residual activity of oxidative enzymes, which may not have have been inactivated by such a low-pressure treatment.

The effect of the food matrix may also affect the efficacy of HHP. Strawberry-based beverages formulated using a milk matrix and treated with HHP, showed a significantly reduced content of anthocyanins, compared with those beverages formulated with a water matrix. This indicates that anthocyanins and milk proteins formed secondary complexes (Tadapaneni et al. 2012). HHP was studied as an anthocyanin extraction method from grape by-products (including skins, stems and seeds) and compared to PEF and ultrasonication treatments. HHP processing (600 MPa at 70 °C for 1 hour) gave 25% lower anthocyanin yields in comparison to the PEF treatment (3 kV/cm, with a series of 30 pulses to obtain 10 kJ/kg), but 40% higher yields compared to ultrasonication (35 kHz) (Corrales et al. 2008).

The sensitivity of various anthocyanins to HHP treatment depends on their structure. Increase in the number of methoxyl and hydroxyl groups on the B-ring led to higher extraction of anthocyanins from grape-skin by HHP treatment (Corrales et al. 2009). The extraction of acylated glucosides of anthocyanin was remarkably higher after HHP treatment, compared to anthocyanin monoglucosides (Corrales et al. 2008; Corrales et al. 2009). The highest levels of anthocyanidin monoglucosides were obtained at low pressures (200 MPa), whereas pressure at 600 MPa was optimal in acylglucoside extraction in grape

skin. An increase in temperatures to over 70 °C did not further increase the extraction yield of acylated anthocyanins, neither did a longer extraction time (Corrales et al. 2009). Long treatments at high temperatures might enhance the condensation reactions of anthocyanins and consequently explain the lower extractability of anthocyanins during HHP treatment. Pelargonidin linked to glucose or malonylglucose sugars were more stable at lower pressures than pelargonidin linked to rutinose or coumaroylglucoside in strawberry based beverages (Tadapaneni et al. 2012). Although pressure and temperature seem to have a synergistic effect on anthocyanin degradation, high pressure treatments can be advantageous in reducing the duration of the treatment, and thus, reduce the overall impact of the processing on phenolic compounds in berry-based products.

There is a lack of studies on berries as regards the influence of high pressure processes on phenolic compounds, other than anthocyanins. However, Cao et al. (2011) studied the impact of pressures of 400–600 MPa for 5–25 min on phenolic compounds in strawberry pulp. The impact was studied on kaempferol, myricetin, quercetin, catechin, caffeic, ferulic, *p*-coumaric, β-hydroxybenzoic and ellagic acids. Among the phenolics studied, catechin was the only compound where a loss of content was observed (around 20%) when a pressure of 400 MPa was used for 10 min. This loss might be due to the residual activity of PPO and POD, which use catechin as a substate. With higher pressures (500–600 MPa) the content of catechin remained stable, probably due to inactivation of the native enzymes (Cao et al. 2011).

2.5 Effect of storage on phenolic compounds in juices, jams and purées of berries

Storage leads to a similar decomposition of phenolic compounds as thermal treatment but the endpoint is highly dependent on the storage temperature, light, presence of oxygen, oxidizing native enzymes, pH, and also the berry species and cultivar (Bimpilas et al. 2015; Holzwarth et al. 2013; Kallithraka et al. 2009; Teleszko et al. 2016; Wicklund et al. 2005; Wilkes et al. 2014). For color retention, lowered storage temperatures, light protection, pectins and a high sugar content in the products have all found to be favorable. In the following chapters, the impact of storage is reviewed on conventional products including clear and cloudy juices, jams, and purées.

Anthocyanins. Anthocyanins of various berry products were retained to much higher extent during cold storage (+4–9 °C) compared to storage at temperatures of 15–37 °C, with a decreasing trend being seen as the time of storage increased (Aaby et al. 2007; Brownmiller et al. 2008; García-Viguera et

al. 1999; Hager et al. 2008; Hellström et al. 2013; Holzwarth et al. 2013; Howard et al. 2010; Oszmiański & Wojdyło 2009; Patras et al. 2011; Teleszko et al. 2016; Wicklund et al. 2005; Wilkes et al. 2014). During storage, the degradation of anthocyanins followed the first-order kinetic model, as was observed during processing (Garzon & Wrolstad 2002; Hellström et al. 2013; Patras et al. 2011; Skrede et al. 1992). Degradation is temperature dependent (Wang & Xu 2007).

Various types of added pectins in berry jams seem to influence the stability of anthocyanins during storage more than the cooking temperatures during the preparation of jam. A low degree of esterification of pectins (LM pectins) enhanced the stability of the pigments in strawberry jam. Amidated pectins (LM pectin obtained from HM pectin when ammonia is used in the alkaline de-esterification process) also increased the pigments stability, possible *via* the formation of additional hydrogen bonds between the hydroxyl groups of the anthocyanins and the amide groups of pectins. Pectin together with high contents of calcium, iron and aluminium were found to improve the stability of the pigment (Holzwarth et al. 2013). This probably resulted from chelate complexes between the metal ions and vicinal hydroxyl groups of anthocyanidins, mainly cyanidin (Castañeda-Ovando et al. 2009; Wrolstad & Erlandson 1973). Native pectins present in purées and cloudy juices seemed to increase the stability of anthocyanins, compared to clear juices. The hydrophobic structure of pectin and cell wall polysaccharides provides natural colloidal suspensions (Hartmann et al. 2008; Oszmiański & Wojdyło 2009). Moreover, pectins might have co-pigmented with anthocyanins, protecting the colored flavylium cation for a longer time during storage. In general, clear juices seemed to suffer the greatest losses of phenolic compounds due to the multiple processing steps and the lack of protective pectins, compared to jams, purées, and cloudy juices.

The effects of additional sugar on anthocyanin stability are controversial. A higher sugar addition to berry jams and frozen strawberries resulted in better pigment retention (de Moura et al. 2012; Wrolstad et al. 1990), associated with the lower water activities (Erlandson & Wrolstad 1972; Holzwarth et al. 2013). On the other hand, sugar additions were found to decrease the stability of anthocyanins in jam (Howard et al. 2010; Hubbermann et al. 2006; Tsai et al. 2005). The strawberry cultivar with the lowest acid content and the highest pH of the jams had the highest degradation rates of monomeric anthocyanins during processing and storage (García-Viguera et al. 1999).

The juice matrix had a major impact on the stability of anthocyanins. Cyanidin derivatives degraded 3–4 times faster in crowberry juice than in chokeberry juice. In black currant juice, cyanidin glucose also degraded much

faster compared to chokeberry juice (Hellström et al. 2013). Acylation of anthocyanins with cinnamic and other organic acids is considered to stabilize the structure during storage and processing, by preventing hydrolysis of the flavylium cation (Bąkowska-Barczak 2005; Teleszko et al. 2016). However, significant demalonation of anthocyanin malonyl esters were observed in strawberry purées (Aaby et al. 2007). Methylation of hydroxyl groups in the B-ring increased the stability of anthocyanins (Hellström et al. 2013). The type of sugar attached to the anthocyanidin had little effect on the stability of anthocyanins in various berry juices (Hellström et al. 2013; Wilkes et al. 2014), which was in contrast to the findings observed in response to thermal treatments (Wilkes et al. 2014).

The anthocyanin degradation during storage of various berry products was followed by increased polymeric color (Brownmiller et al. 2008; Hager et al. 2008; Howard et al. 2010; Poiana et al. 2012; Wilkes et al. 2014). The dark purple color of chokeberry juices seemed to be well retained, despite major losses in monomeric anthocyanins, indicating possible co-pigmentation reactions in color stabilization. As color polymerization is already started during processing, it is therefore possible that the polymers were more resistant to degradation during storage (Wilkes et al. 2014). A higher content of polymeric pigments were obtained with higher storage temperatures and light illumination of strawberry jams (Holzwarth et al. 2013). Supplementary low esterification and amidated pectins led to lower polymeric color values (Holzwarth et al. 2013). Sugar seemed to promote the formation of polymers by lowering the water activity and pH (Howard et al. 2010).

Flavonols. Flavonols as free aglycones and as sugar derivatives were commonly considered highly stable during storage. The levels of total flavonols were unaffected by storage time, temperature or sugar addition in strawberry jams (Howard et al. 2010; Häkkinen et al. 2000a). During the storage of berry juices, the increase in free flavonols was accompanied by a decrease in the corresponding glycosides (Aaby et al. 2007; Häkkinen et al. 2000a; Laaksonen et al. 2012; Teleszko et al. 2016; Zafrilla et al. 2001). Hydrolysis of flavonol glycosides increased with the higher storage temperatures of strawberry cloudy juice and raspberry jam (Teleszko et al. 2016). Wilkes et al. found a decrease (20–30%) in quercetin glycosides and aglycone in chokeberry juice, with reference to the oxidation of flavonols into quinones, after a storage period of four months at 25 °C. Since the juice was pressed with supplementary pectinase, the physical binding of flavonols into soluble solids is not likely to be the reason for the decrease (Wilkes et al. 2014).

Tannins. Storage at 25 °C for 6 months resulted in marked losses (around 30%) of procyanidins in the juices, purees and jams of blueberries

(Brownmiller et al. 2009; Howard et al. 2010). However, the decrease of procyanidins during storage was of a lesser extent compared to the impact of thermal processing on blueberry juices. Mono- and dimers showed greater losses during storage of blueberry purées than during the processing of the purées (Brownmiller et al. 2009). In chokeberry juice, the levels of total proanthocyanidins retained more stable compared to blueberry products (Brownmiller et al. 2009; Wilkes et al. 2014), which indicated that the proanthocyanidins did not react with anthocyanins to form polymeric pigments during storage.

Oligomers of a lower DP were retained to a higher extent than oligomers of a higher DP in blueberry juices and jams. The losses were most likely derived from polymerization reactions with anthocyanins and/or binding to the proteins and cell wall polysaccharides, thus forming a precipitate during storage (Brownmiller et al. 2009; Howard et al. 2010). Cold storage retained the oligomers to a higher level compared to storage at 25 °C (Howard et al. 2010). It is possible that the procyanidins were degraded by native oxidizing enzymes, which were not completely inactivated by the blanching treatment (Goupy et al. 1995; Sang et al. 2004).

(+)-Catechin was found to be very labile during storage of strawberry purée. The decrease might be explained by partial thermostable oxidixing enzyme POD, of which (+)-catechin is a major substrate (López-Serrano & Barceló 1996; López-Serrano & Ros Barceló 2002). Similarly to the anthocyanins, the native pectins in cloudy juices and purées seem to stabilize the polymeric proanthocyanidins, compared to these compounds in clear juices (Oszmiański & Wojdyło 2009; Teleszko et al. 2016).

During storage at room temperature, the content of ellagic acid increased in raspberry jam and strawberry purée (Aaby et al. 2007; Zafrilla et al. 2001). Furthermore, in raspberry jams an initial increase in the ellagic acid content was observed during the first month of storage, and a decrease in the following three months of storage (Zafrilla et al. 2001). The content of ellagic acid also increased significantly in cold storage (+4 °C) in strawberry cloudy juices and purées, but to a lesser extent compared to higher storage temperatures (Oszmiański & Wojdyło 2009; Teleszko et al. 2016).

Ellagic acid glycosides, on the other hand, were not to any considerable extent affected by the room temperature storage of raspberry jam and blackberry clear juices and purées (Hager et al. 2010; Zafrilla et al. 2001). However, ellagic acid glycosides decreased significantly in strawberry purée and blackberry cloudy juices, and more so at higher temperatures (Aaby et al. 2007; Hager et al. 2010). The greater losses of ellagitannins in cloudy juices in comparison to the clear juices of blackberries might be due to the binding of

the compounds to insoluble materials such as cell wall polysaccharides and/or proteins.

Phenolic acids. Loss in *p*-coumaroyl sugar derivatives was noted during storage at +4 °C in cloudy strawberry juice and purées. The loss was significantly higher during storage at 20 °C (Aaby et al. 2007; Teleszko et al. 2016). Levels of phenolic acids were found relatively stable during storage at room temperature (Hellström et al. 2013; Howard et al. 2010; Wilkes et al. 2014). The contents remained unchanged during up to three months of storage, and then declined by about 30% from 3–6 months of storage in chokeberry juice. This finding indicated that anthocyanins were not degraded into hydroxybenzoic acids during storage (Wilkes et al. 2014).

During storage, only quercetin in strawberry purée followed the first-order kinetic rate. The change in the contents of kaempferol, *p*-hydrozybenzoic acid and ellagic acid was non-linear (Marszałek et al. 2016). This non-linear trend of the compounds might be a result of the release of these compounds through hydrolysis of their derivatives, and/or degradation of anthocyanins (Amakura et al. 2000; Marszałek et al. 2015; Odriozola-Serrano et al. 2008b; Prior et al. 2001; Wilkes et al. 2014). Moreover, random binding to cell wall polysaccharides or polymerization/co-pigmentation with anthocyanins/ proanthocyanins may also influence the final content of the phenolic compounds in berry products.

2.6 Concluding remarks

The extent of the decomposition of phenolic compounds depends on the types of process, process parameters, storage conditions, the species and cultivars of the berries, the matrix of the berry products, and the chemical reactions of phenolic compounds with other compounds in the matrix. Presence of oxygen and oxidizing native enzymes, as well as the supplementary enzymes, pectins and sugars may also play a role.

During conventional thermal processing and storage at room temperature, anthocyanins suffer great losses in the berry products. The loss in monomeric anthocyanins is accompanied by an increase in percentage polymeric color values through the formation of an anthocyanin-tannin/flavanol complex. Polymerization reactions in berry products seem to take place with other forms of anthocyanidins than the flavylium cation resulting in colorless or brownish pigments. Co-pigmentation often results in stabilization of the flavylium cation in the complex stabilizing the color. However, the co-pigmentation reactions during processing and storage are less studied in berry products.

Flavonols are generally well retained during processing. However, significant quantities of flavonol aglycones might be released during the thermal processing of berry products. Processing often leads to conversion of proanthocyanidin oligomers into monomers, dimers, and trimers. Proanthocyanidins with a high degree of polymerization (DP) are poorly absorbed relative to their monomeric subunits. Thus, certain heat treatments might be able to increase the bioavailability and absorption in the human intestine system by decreasing the average DP. During processing, proanthocyanidins may form polymers with anthocyanins and/or bind to other polymers in the cell wall, the latter resulting in precipitation during storage. The levels of free ellagic acid and phenolic acids have been found to increase during thermal processing. These phenomena could be explained as the spontaneous hydrolysis of bounded forms into free compounds, as well as a more efficient extraction of these compounds, and/or a degradation of the anthocyanins.

The more unconventional applications of hydrothermodynamic processing, microwave heating, pulsed electric field treatment and high pressures were found useful in improving the extraction yields and reducing the decomposition of phenolic compounds in the final berry product. Cavitation and electroporation increase cell membrane disintegration of the berry tissues, resulting in a high content of phenolic compounds in the berry product. High pressure also causes berry cell disruption, but it mainly affects noncovalent bonds, thus preserving the color of the final products well. The radiant energy of microwaves heats the product directly, which makes it possible to treat higher-density berry products with shorter treatment times. In order to commercialize the more unconventional processes for berries, there is a need for consumer education to overcome possible prejudices and to gain consumer acceptance.

The research at the moment, concerning the processing technologies of berries, has mainly concentrated on studying the content of total phenolics and anthocyanins. There is a clear lack of investigations on other important bioactive phenolics, such as other flavonoids, phenolic acids and tannins, which exist in considerable amounts in berries, and have an enormous impact on the bioactive properties and the flavor of the berry products. Much of the research reviewed is carried out in model systems, which may not represent the situation in real food systems. More studies are needed to investigate the stability of phenolic compounds in berry products as the outcome of complex interaction between the food matrix, the processing technologies and the storage conditions.

3 AIMS OF THE EXPERIMENTAL STUDY

The overall aim of the research was to study the impact of various process technologies and storage conditions on the chemical and sensory characteristics of black currant based products.

The first aim was to identify and quantify flavonoids, phenolic acids, sugars and non-phenolic fruit acids and to study their behavior during various berry pressing processes and storage at various conditions. Special focus was placed on the less investigated hydroxycinnamic acids in order to verify the molecular structure and decomposition (**I**, **III**, **IV**).

The second aim was to study the sensory characteristics of various black currant juices from various cultivars (**I**), to investigate the impact of processing technologies (**I**, **IV**) and to follow the changes of sensory properties during storage at various conditions (**IV**).

The third aim was to develop extrusion processing for the press residue from the berry pressing processes. The impact of varying press residues and cereal materials was investigated on the physico-chemical properties of extrudates (**II**).

4 MATERIALS AND METHODS

4.1 Sample materials

Black currant berries of cultivars 'Mortti' (**I**, **III**, **IV**), 'Mikael', 'Marski' and 'Ola', and 'Breed 15' (**I**) were obtained from Southern Finland (the Natural Resources Institute Finland (Luke) Piikkiö, Finland) and harvested in August 2010. The berries were stored in a freezer (–20 °C) immediately after harvesting.

'Mortti' ('Öjebyn' x 'Wellington XXX') is the most cultivated black currant in Finland. The berries are well suited to picking using modern mechanical techniques, and are winter hardy. The skin of the berry is fairly thick. The berries of 'Mortti' are standard black currants for industrial processing.

'Marski' ('Mortti' x 'Hedda') is a quite new cultivar. The blooming takes place at the same time or earlier than 'Mortti'. The crop is somewhat weaker than that of 'Mortti', but the berries are bigger.

'Mikael' ('Brödtorp, self-pollinated' x 'Brödtorp, self-pollinated') was registered by the Natural Resources Institute Finland (Luke) Piikkiö, Finland in the year 2010. The blooming of the young bushes takes place at the same time as 'Mortti', but for older bushes it is a little earlier. It has an ample crop. The size of the berry is similar to 'Mortti'.

'Ola' ('Wellington XXX' x 'Lepaan musta') has an abundant crop. The berry bunches are long, and the berries are middle sized. The harvesting time for the berries is fairly late.

'Breed 15' ('Risager self-pollinated') has bushes lower than those of 'Mortti'. The crop is more abundant and winter hardy, compared to other black currant cultivars in the study (Ahosen Taimisto Oy, 2016).

Black currants of the cultivar 'Mortti' (**II**), crop 2011, were provided by Saarioinen Oy (Huittinen, Finland) and separated from the stems and leaves by Toripiha Oy (Vesanto, Finland). The black currants were pressed by Saarioinen Oy (Huittinen, Finland). The press residues collected from non-enzyme-aided berry pressing and traditional enzyme-aided berry pressing (**Figure 7**) were stored in a freezer (–20 °C) for further processing. The cereal material of oat, barley and potato flour, oat bran, and potato flakes were obtained from Leipomo Rosten Oy (Turku, Finland). The salt and sugar used were basic commercial products (**II**).

4.2 Sample processing

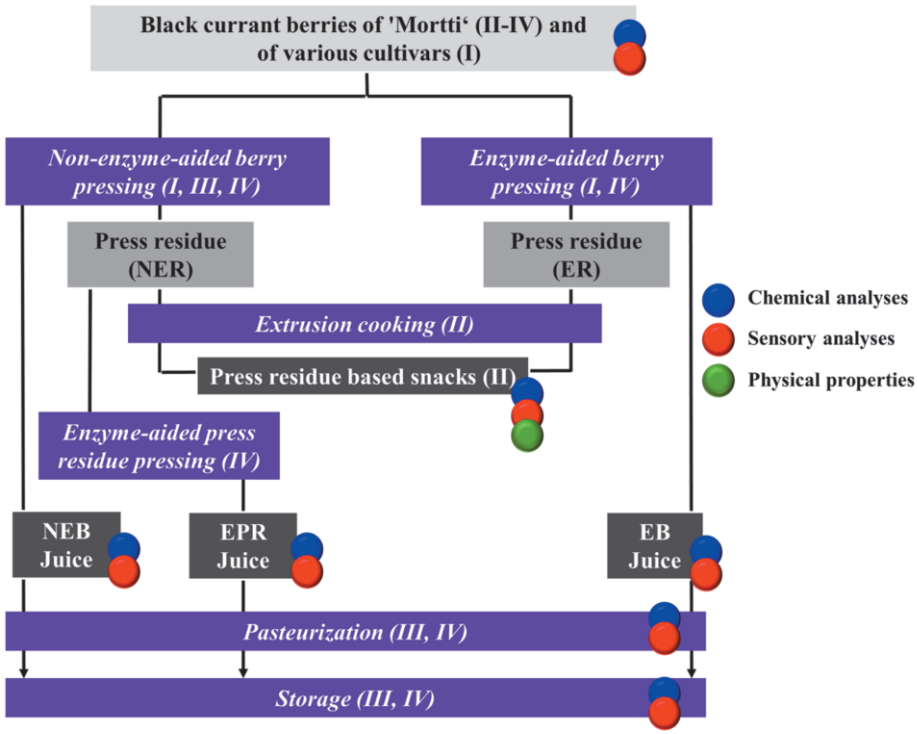

Figure 7. Overall scheme of the processes (in violet boxes) and analyses (colorful circles) conducted in the study.

4.2.1 Berry pressing processes

Berries were pressed with and without the application of macerating enzymes with a hydraulic juice extractor (**Figure 7**) in laboratory scale. The non-enzymatic pressing yielded "Non-Enzymatic Berry" juice (NEB-juice) with juice yield of 60% (**I**, **III**, **IV**). In the enzyme-aided pressing, berries were macerated with supplementary enzyme product (Pectinase 714L, purchased from Biocatalysts Ltd. (Cardiff, UK), containing pectinase (< 33%), polygalacturonase (< 33%), and β-glucanase (< 33%) activities), and pressed to yield "Enzymatic Berry" juice (EB-juice), i.e. the conventional berry juice (**I, IV**). The press residue of 'Mortti' was collected from the NEB-juice pressing and frozen (–20 °C). A batch of the press residue was thawed, macerated with supplementary enzyme and pressed to yield "Enzymatic Press Residue" juice (EPR-juice) (**IV**). The enzyme incubation was conducted at 45 °C for 4 hours.

After pressing, the juices were pasteurized in batches at 95–97 °C for 30 seconds (**III, IV**).

In the one-year storage test, samples were stored in a refrigerator (+4 °C) or at room temperature either in the dark or exposed to surrounding light (**III**, **IV**). Sample collection was done before and after pasteurization (Reference I and II) and at the time points of 3, 6, 9 and 12 months. Possible growth of bacteria, yeast and molds in the juices was tested at the time of collection. No growth was detected in any of the samples. All the samples were stored in the freezer for further analyses (**III**, **IV**).

4.2.2 Extrusion processes

Non-enzymatically treated berry press residue (NER) and berry press residue from the conventional enzyme-aided pressing (ER) were chosen for the raw materials in the production of extrudates (**II**). Prior to extrusion, the press residues were air-dried at 40 °C in drying-cabinets, NER for ~13 days and ER for ~7 days until the weight no longer decreased from the drying process. ER was properly milled to break down the seeds and sieved through a sieve with a 0.5 mm screen. NR was crushed into coarse particles with a mortar, and the largest particles removed with a domestic sieve.

Press residues were processed with six different recipes, three recipes for each press residues. The recipes consisted of NER/ER (content of 27–28%), barley flour/oat flour/oat bran (38–39%), potato flour (14–15%), potato flakes (14–15%), sugar (4.7–4.8%) and salt (0.5%). The extrudates were produced with a twin-screw extruder, with the following parameters: meal feeding rate 4.9–7.0 kg/h, water feeding rate 350–730 mL/h and screw speed 400–420 rpm. The temperatures of the first two heating units were kept at a constant 95 °C. The barrel temperature of the last heating module varied between 94–102 °C. The screw torques varied between 13–16 Nm, and the bearing pressure between 55–120 bars. Extrudates were sealed in plastic bags filled with nitrogen to generate a modified atmosphere and remove the oxygen (**II**).

4.3 Chemical and physical analyses

Phenolic acids and flavonols were extracted with ethyl acetate by consecutive procedures. The compounds were identified by a reverse phase high performance liquid chromatography coupled with DAD (**I**, **III**, **IV**), ESI–MS(–MS^2) (**III**, **IV**) and Q-TOF-MS (**III**), with the help of reference compounds and literature data. The structures of the selected compounds were confirmed with ^1H and ^{13}C NMR after purification with Sephadex LH-20 gel and preparative HPLC fractionation (**III**). In HPLC–DAD–ESI–MS(–MS^2) analysis, the mass

spectrometer operated simultaneously in positive and negative ion modes for the MS-data, and separately for the MS^2-data. Full scan mass spectra (MS) were obtained by scanning ions between *m/z* 80 and 1000. Chromatograms were recorded by scanning the absorption at 190–600 nm, monitoring hydroxycinnamic acids at 320 nm, flavonols at 360 nm, and hydroxybenzoic acids at 250 or 290 nm. Citric acid methyl esters and flazin were monitored at 290 nm and 320 nm, respectively. The reference compounds of the hydroxycinnamic acids were irradiated with a led-light (one hour) emitting UV-A radiation (360–370 nm) with an intensity of 41 W/m^2. The reference compounds were also irradiated under sunlight for seven days in August, 2015 (**III**).

For structure determination of the selected compounds, raw ethyl acetate extracts were purified with Sephadex LH-20 gel to remove unwanted sugars and to obtain fractions with higher concentrations of the selected compounds. Semi-preparative HPLC was used for isolation of the pure selected compounds. ^1H and ^{13}C NMR spectra were obtained from the pure compounds using 1D-TOCSY, HSQC, COSY and HMBC measurements as additional tools, to determine the detailed structure of the purified compounds (**III**).

For anthocyanin analyses, samples were prepared by extracting the juice with acidified methanol (Sandell et al. 2009). The quantification of the analytes was carried out by HPLC–DAD using the calibration curves constructed with external standards (**I**, **III**, **IV**) (Laaksonen et al. 2012; Sandell et al. 2009; Zheng et al. 2012). The standards were either purchased or purified reference compounds of the corresponding analyte. See Table 2 for the list of reference compounds used for the phenolic acids, citric acid methyl esters and flazin.

The sugars and non-phenolic organic fruit acids in the black currant juices were analyzed based on a previous method (Zheng et al. 2009) (**I**, **IV**). Sugars and non-phenolic organic acids, as well as the protein, crude fat, moisture and ash content in the extrudates were analyzed in the test laboratory T024, accredited by the Finnish Accreditation Service – FINAS at the Natural Resources Institute Finland – Luke. Energy and carbohydrates (all the carbohydrates which are metabolized in the human body) were defined computationally based on the recipes. The total amount of fiber was determined enzymatically with BIOQUANT® Total Dietary Fiber Method (AOAC International, 1995). In the fiber analysis a crude protein analysis was also carried out, applying the Kjeldahl method. Additionally, NDF-fiber and ADF-fiber analyses were conducted; hemicellulose was calculated as NDF-ADF. Amino acids were detected by ion exchange chromatography with a ninhydrin post column derivatization using commercial calibration standards (**II**).

Extrudates were measured by length, height, mass, sectional expansion index (SEI), bulk density, WAI (water absorption index), WSI (water solubility index) and hardness. The color of the extrudates was measured using color space parameters *L**, *a**, *b** (**II**).

Table 2. The reference compounds used for quantification of the phenolic acids, citric acid methyl esters and flazin.

Analytes	***Reference compounds used in the quantification***
Hydroxycinnamic acids	
neochlorogenic acid	(*E*)-caffeic acid
(*E*)-caffeic acid *O*-glucoside and (*E*)-*p*-coumaric acid *O*-glucoside [1]	(*E*)-caffeic acid
(*E*)-caffeoylglucose	(*E*)-caffeic acid
(*E*)-*p*-coumaroylquinic acid	(*E*)-*p*-coumaric acid
(*Z*)-*p*-coumaric acid *O*-glucoside	(*E*)-*p*-coumaric acid
(*E*)-*p*-coumaroylglucose and chlorogenic acid [1]	(*E*)-*p*-coumaric acid
(*Z*)-*p*-coumaroylglucose	(*E*)-*p*-coumaric acid
(*E*)-caffeic acid	(*E*)-caffeic acid
(*E*)-feruloylglucose	(*E*)-ferulic acid
(*E*)-*p*-coumaric acid	(*E*)-*p*-coumaric acid
(*Z*)-*p*-coumaric acid	(*E*)-*p*-coumaric acid
(*E*)-ferulic acid	(*E*)-ferulic acid
(*E*)-caffeoyloxymethyleneglucosyloxy-butenenitrile	purified compound of (*E*)-coumaroyloxymethylene-glucosyloxybutenenitrile
(*E*)-coumaroyloxymethyleneglucosyloxy-butenenitrile	purified compound of (*E*)-coumaroyloxymethylene-glucosyloxybutenenitrile
(*Z*)-coumaroyloxymethyleneglucosyloxy-butenenitrile	purified compound of (*E*)-coumaroyloxymethylene-glucosyloxybutenenitrile
(*E*)-feruloyloxymethyleneglucosyloxy-butenenitrile	purified compound of (*E*)-coumaroyloxymethylene-glucosyloxybutenenitrile
Hydroxybenzoic acids (anthocyanin degradation products)	
protocatechuic acid	protocatechuic acid
4-hydroxybenzoic acid	4-hydroxybenzoic acid
phloroglucinaldehyde	phloroglucinaldehyde
Citric acid methyl esters and flazin	
citric acid methyl esters	(*E*)-*p*-coumaric acid
flazin	purified compound of flazin

[1] When the sample peaks overlapped, the reference compound was chosen for the more abundant analyte.

4.4 Sensory and hedonic response (liking) analyses

Sensory and hedonic tests were conducted in controlled sensory laboratory conditions in accordance with the ISO8589 standard. The sensory characteristics of the juices of various black currant cultivars (**I**), various processes (**I**, **IV**) and those stored at room temperature in the dark and at +4 °C (**IV**) were analyzed using generic descriptive analysis. Trained (ISO 8586-1) panelists evaluated the sweetness, sourness, bitterness, puckering astringency, mouth-drying astringency, total intensity of flavor, berryness, aftertaste and roundness with the help of reference samples (**I**, **IV**). The first five attributes were chosen in order to evaluate the juices of the storage trial (panel, n = 11) (**IV**), since these attributes were found to correlate with the content of phenolic compounds. The aim was to analyze NEB-juices (panel, n = 14) and EB-juices (panel, n = 13) of the various black currant cultivars (**I**), and study the influence of pasteurization (n = 14) on NEB-juices (**IV**). The intensities of the attributes were rated on a continuous graphical scale from 0 (none) to 10 (extremely strong). Compusense-five software was used for data collection.

The change in color intensity of the juices during pasteurization and storage was assessed (panel, n = 11) (**IV**). The subjects ranked the samples in a randomized and blind coded manner by the intensity of the color using a scale from 1 (the lightest intensity of color) to 6 (the highest intensity of color). Thawed black currant berries served as a reference for the highest intensity of the color.

In the hedonic test, a panel of 77 voluntary subjects rated their liking of the properties of the extrudates (**II**). Appearance (shape and color), flavor, texture and the overall pleasantness of the six samples were measured in a randomized and blind coded manner on a nine-point balanced hedonic scale. Additionally, the subjects ranked the six samples in the order of preference (ISO 8587).

4.5 Statistical analyses

Samples were studied by the analyses of variance (ANOVA) together with a post hoc test (Tukey's, Tamhane or LSD test) or a t-test. A one-way ANOVA was used to compare the chemical variables of the cultivars within each berry pressing process (**I**), storage time (**III**) and storage condition (**III**, **IV**). A student's t-test was used to study the differences in chemical and sensory variables between the two juice processing technologies (**I**). A paired samples t-test was used to study the effect of pasteurization and storage (pasteurized sample vs. 12-month storage sample) (**IV**).

The sensory results were analyzed with three-way ANOVA test with juice samples as fixed factors, and panelists and sessions as random factors (**I**, **IV**). Hedonic response data of the extrudates was analyzed with a two-way ANOVA (sample, gender) (**II**). Friedman's analysis of variance was used for the ranking of extrudates (**II**). The ranking for the color intensity of the juices was analyzed using Friedman's Analysis of Variance and a Wilcoxon Signed-Rank Test (**IV**). The criterion for statistical significances was $p < 0.05$. ANOVA models and *t*-tests were performed using SPSS software.

The influence of various processing technologies on chemical properties of juices and physical properties of extrudates were studied by applying principal component analyses (PCA) to the standardized data (**II**, **IV**). A partial least squares regression (PLS) model was used to find relationships between the chemical and sensory data matrices (**I**, **IV**), and between liking and physico-chemical properties (**II**), which were applied to the standardized data. X-variables (predictors) were the chemical or physico properties and Y-variables (responses) were the sensory or hedonic responses. PCA and PLS models were executed with Unscrambler software.

5 RESULTS AND DISCUSSION

5.1 Identification of hydroxycinnamic acids

One of the main interests of the thesis was to determine the structure and stability of the less investigated hydroxycinnamic acids in black currant juices. To some extent, the HCAs were already identified in study **I**, but a more detailed identification was conducted in study **III**. The HCAs were verified as derivatives of *p*-coumaric, caffeic and ferulic acids (**Figure 8**). Derivatives of *p*-coumaric acid dominated in black currant juice, while derivatives of ferulic acid occurred in lower concentrations and with a smaller number of compounds. Previously identified 1-*O*-acylated glucoses of the HCAs (compounds no **3**, **6** and **9**, **Figure 8C** and **9**) (Koeppen & Herrmann 1977; Lu & Yeap Foo 2003) were identified based on the bathochromic shift of the absorption maxima in the UV-spectra compared to the spectra of the corresponding free HCAs (Määttä et al. 2003). The compounds shared a similar MS^2 fragmentation pattern of $[M + H - glc - H_2O]^+$ in the positive ion mode of ESI.

Nitriles of HCAs (**Figures 8E** and **F** and **Figure 9**) had odd numbered nominal masses [M] of *m/z* 437, 421 and 451. The compounds shared a similar fragmentation pattern; caffeic (*m/z* 180) / coumaric (*m/z* 164) / ferulic acid (*m/z* 194) + *m/z* 162 (glc) + *m/z* 95. Moreover, for every nitrile compound the MS^2 spectrum showed a positive ion fragment with *m/z* value of 114 $[M + H - (HCA - H_2O) - glc]^+$. The structure determination of 2-(*E*)-*p*-coumaroyloxymethylene-4-β-D-glucopyranosyloxy-2-(*Z*)-butenenitrile (no **14**) and 2-(*Z*)-*p*-coumaroyloxymethylene-4-β-D-glucopyranosyloxy-2-(*Z*)-butenenitrile (**15**) revealed the nitriles as geometric isomers. The remaining nitriles were identified as 2-(*E*)-caffeoyloxymethylene-4-β-D-glucopyranosyloxy-2-(*Z*)-butenenitrile (**13**) and 2-(*E*)-feruloyloxymethylene-4-β-D-glucopyranosyloxy-2-(*Z*)-butenenitrile (**16**).

Tentatively identified *O*-glucosides (Määttä et al. 2003; Schuster & Herrmann 1985; Schwarz & Hofmann 2007) of (*E*)-*p*-coumaric and (*E*)-caffeic acids co-eluted in the HPLC chromatogram (**Figure 8G** and **Figure 9**). The 4-*O*-glucosides were identified based on similar MS and MS^2 spectra as the corresponding 1-*O*-acylglucoses, and also from the elution before the corresponding 1-*O*-acylglucose (Anttonen & Karjalainen 2006). The 4-*O*-glucosides showed hypsochromic shift (change of the absorption spectra towards shorter wavelengths) in the UV-spectra compared to the free HCA reference compound (Anttonen & Karjalainen 2006). Previously identified *O*-

acylquinic acids were also detected (**Figures 8I** and **J** and **Figure 9**) (Anttonen & Karjalainen 2006; Czyzowska & Pogorzelski 2014).

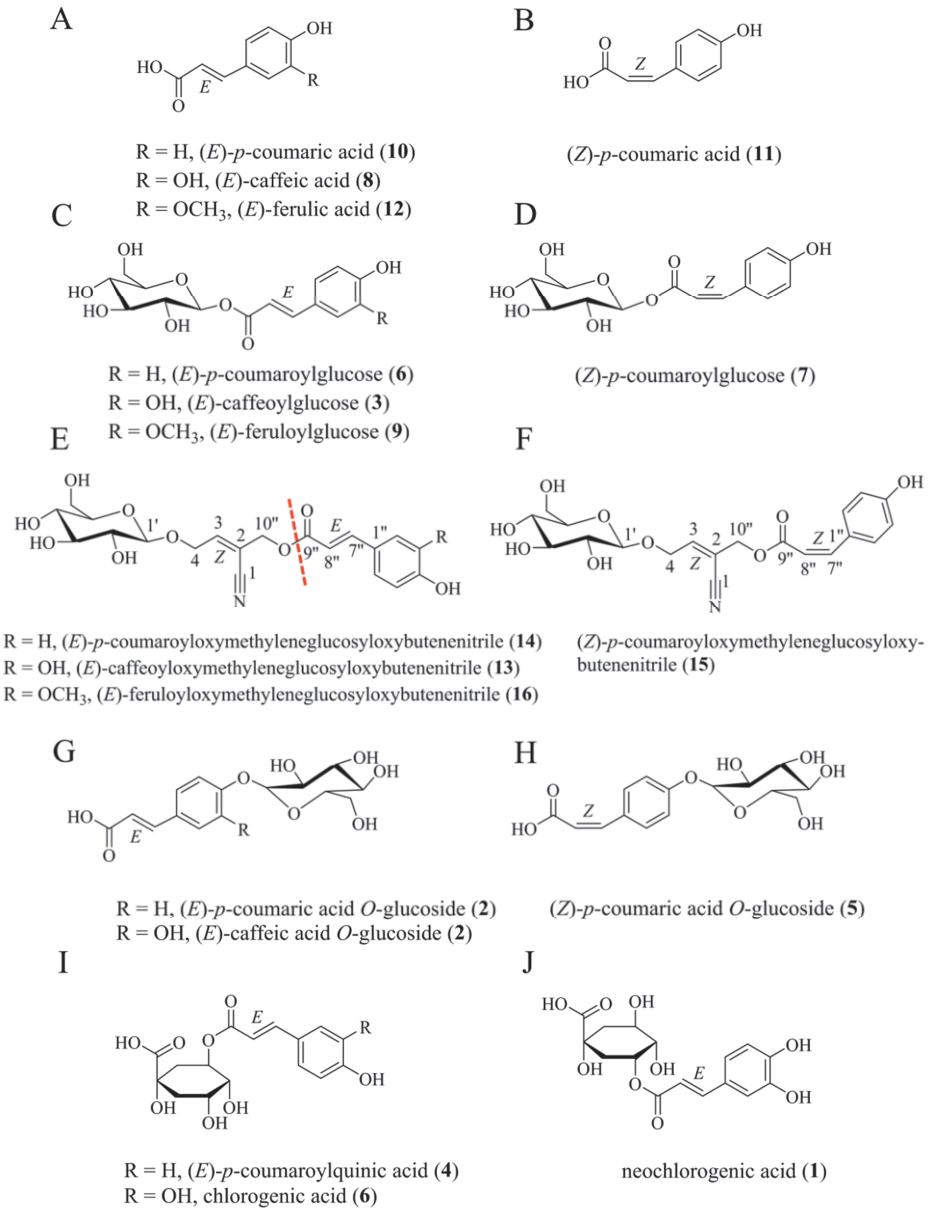

Figure 8. Hydroxycinnamic acids examined in the study (**III**). The red dotted line indicates a probable position for hydrolysis of *O*-acyl derivatives of HCAs. The numbers refer to chromatographic retention in the **Figure 9**.

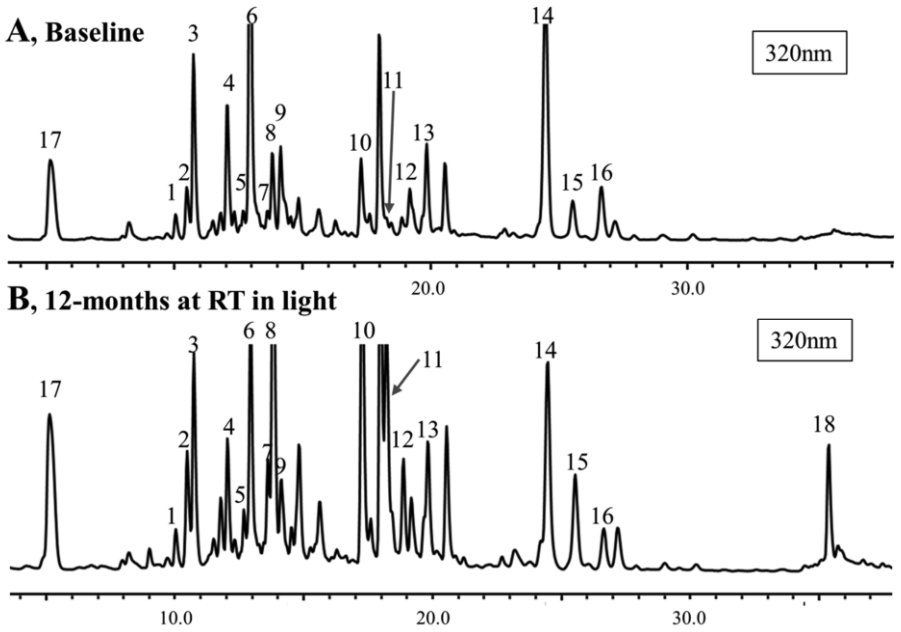

Figure 9. Chromatograms of ethyl acetate extract of hydroxycinnamic acids (**1–16**), and citric acid methyl esters (**17**) and flazin (**18**) in NEB-juice, obtained *via* HPLC–DAD (**III**). A) Baseline sample (after pasteurization), and B) sample stored for one-year at room temperature (RT) in light. An Aeris PEPTIDE XB-C18 (150 mm × 4.60 mm i.d., 3.6 µm) analytical column was used, attached to a guard column (2.00 mm × 4.60 mm i.d.) (Phenomenex, Torrance, CA). Eluent A was 0.1% formic acid in Milli-Q water, and eluent B was 0.1% formic acid in acetonitrile. The gradient program for eluent B: 0−15 min, 2−18%; 15−20 min, 18%; 20−30 min, 18−20%; 30−35 min, 20−60%; 35−40 min, 60−2%; and 40−45 min, 2%. The structures of the numbered compounds **1**–**16** are presented in **Figure 8**.

Z-isomers of *p*-coumaric acids (**Figures 8B, D, F** and **H**) eluted after the corresponding *E*-isomers (**Figure 9**), and had similar MS and MS^2 spectra, with a hypsochromic shift in the UV spectra compared to (*E*)-isomers. Free (*Z*)-*p*-coumaric acid was identified with a reference compound created by irradiation of (*E*)-*p*-coumaric acid. Irradiation of (*E*)-*p*-coumaric acid with UV-A light and sunlight led to a partial conversion into the *Z*-isomer (**Figure 10A**). *Z*-isomers of caffeic and ferulic acids in a free form or as derivatives were not detected in the juice samples. Compared with (*E*)-*p*-coumaric acid, (*E*)-caffeic acid converted to the least extent into *Z*-isomer (**Figure 10C**). Therefore, the

second OH-group in the benzene ring seemed to stabilize the system under the stress factors of irradiation with UV and sunlight.

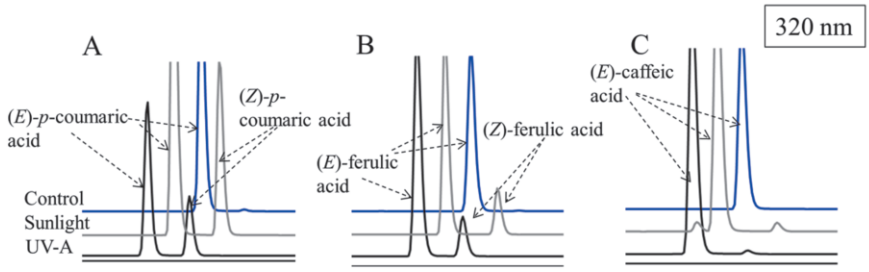

Figure 10. Chromatograms of irradiated reference compounds of A) (*E*)-*p*-coumaric acid, B) (*E*)-ferulic acid and C) (*E*)-caffeic acid, analyzed by HPLC−DAD (**III**).

5.2 Identification of other metabolites

Anthocyanins and flavonols were identified based on the results of previous studies (**I**, **III**, **IV**) (Laaksonen et al. 2012; Sandell et al. 2009; Zheng et al. 2012). Peaks no **17** and **18** (**Figure 9**) were of interest, since the peaks did not belong to the group of phenolic acids, flavonols or anthocyanins based on the UV absorption spectra (**III**). Peak no **17** was formed during pasteurization. The peak **18** increased by approximately 600% during storage at room temperature with only trace amounts in the baseline sample (**Figure 9A**). These metabolites were elucidated by NMR, after purification with semi-preparative HPLC. The peak **18** needed acid in the eluents for proper chromatographic separation during isolation with semi-preparative-HPLC. The compound was verified as flazin [1-(5-hydroxymethyl-2-furyl)-β-carboline-3-carboxylic acid] (**Figure 11A**). When isolated with a semi-preparative-HPLC, peak no **17** consisted of two overlapping compounds, citric acid methyl esters: 2-hydroxy-1,2,3-propanetricarboxylic acid-1-methyl ester (non-symmetric 84%, **Figure 11B**) and 2-hydroxy-1,2,3-propanetricarboxylic acid-2-methyl ester (symmetric 16%, **Figure 11C**).

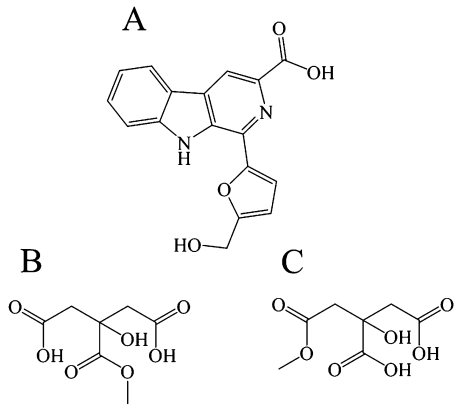

Figure 11. Structures of A) flazin and B) non-symmetric and C) symmetric citric acid methyl esters (**III**).

5.3 Influence of processing

5.3.1 Berry juice processing

5.3.1.1 Berry pressing

The enzyme-aided pressing of berries (EB) elevated the juice yield by 10–20% (Table 3) compared with the non-enzyme-aided (NEB) pressing. Enzymatic pressing of the press residue collected from the NEB-juice pressing yielded 30% of residual EPR-juice. The highest yield among the cultivars was obtained from the berries of 'Mikael' during NEB- and EB-juice processing, due to the low viscosity of the juice.

Table 3. Juice yields (mass-%)[1].

	Mortti	***Mikael***	***Marski***	***Breed 15***	***Ola***
NEB	64 ± 1	70 ± 2	65 ± 3	62 ± 4	59[2]
EB	71 ± 2	77 ± 2	74 ± 1	72 ± 1	72 ± 3
EPR	32 ± 3	-	-	-	-

[1] Mass of the juice in relation to mass of the berries or enzyme-treated berry mash into the pressing unit. [2] Only one pressing was conducted.

5.3.1.2 Influence of processing on the chemical and sensory characteristics of juices

PCA models were applied to combine the data from studies **I**, **III** and **IV** (**Figure 12A**). The aim was to reveal the most influential factors on the metabolic composition and sensory profile of the juices. However, the hydrolysis and degradation products of the phenolics were excluded from the models, since those metabolites were present only in the storage samples (**I**, **III**, **IV**). The influence of storage is discussed in paragraph 5.4.2. The first two validated principal components explained 80% of the variance in the chemical data. A PLS-regression model (**Figure 12B**) was applied to observe the influence of various cultivars, processes and storage conditions and the parameters of the chemical and sensory characteristics (X-data n = 10, Y-data n = 5). In the model, 86% of the chemical variables explained 74% of the variation within the sensory data (R^2 = 0.741; validated R^2 = 0.623, with two factors).

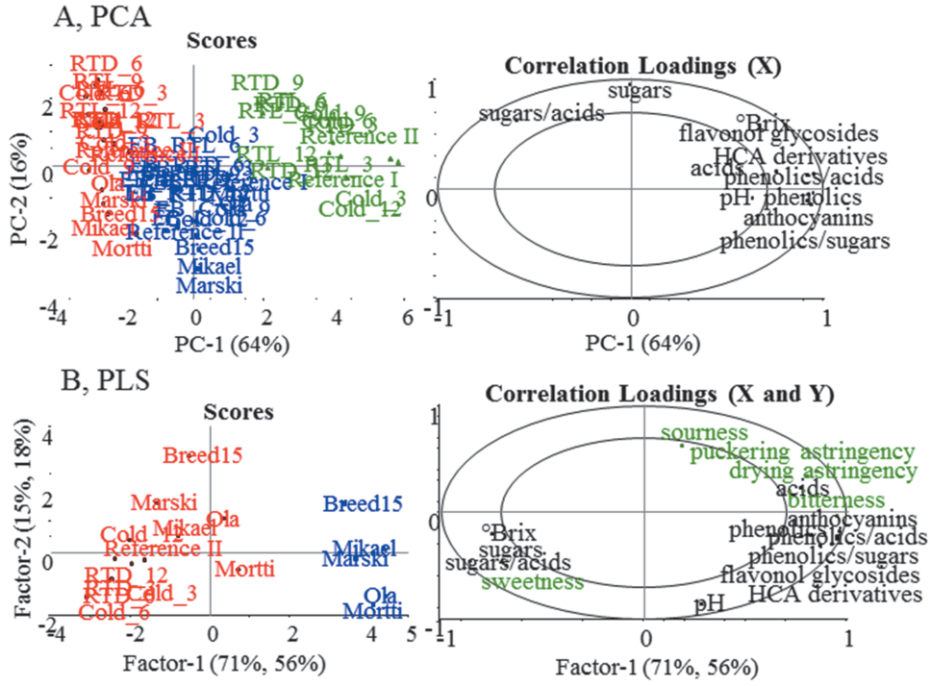

Figure 12. PCA and PLS models illustrating the effect of various berry juice processes, cultivars and storage on black currant juices (**I, III, IV**). A) PCA model of black currant juices (scores, n = 54) and chemical variables (loadings plots, n = 11). B) PLS-regression model of the interactions between chemical and sensory properties (X-data n = 10, Y-data n = 5) of NEB-juices. RTL,

storage at room temperature in light; RTD, storage at room temperature in dark; Cold, storage at +4 °C for 3, 6, 9 and 12 months. Reference I and Reference II: before and after pasteurization of the juices, respectively. NEB-juices in red, EB-juices in blue and EPR-juice in green letters. Loadings of total contents of chemical compounds in black, sensory properties in green letters.

In the first PC of the PCA model (**Figure 12A**), various juice pressing processes were grouped separately from each other. The berry pressing process seemed to have a more significant impact on the chemical composition, compared to the impact of cultivars, pasteurization and storage, which did not separate from each other within each type of processing technology. EPR-juice clearly stood out from the juices from the other two processes due to having the highest content of phenolic compounds and the strongest correlation between sensory properties and phenolic compounds. The raw material used for EPR-juice pressing was the richest in berry skin where the phenolics are mostly located. NEB-juice, located on the left side of the model, correlated negatively with the content of the phenolic compounds. The NEB-juice mostly contained the pulp fraction of the berry, which had the highest amount of sugars and organic fruit acids. The EB-juice located in the middle of the model, combine the characteristics of NEB- and EPR-juices.

The second PC distinguished the juices of different cultivars from the storage samples, indicating that storage had changed the chemical composition of the juices compared to freshly pressed juices. The third PC (data not shown) did not show any additional information in comparison to the second PC. The fourth PC (data not shown) showed mainly the distinctiveness of 'Breed 15' from all of the other juice cultivars, due to the strong correlation with non-phenolic organic acids in the juices.

In the PLS-model (**Figure 12B**), the first factor distinguished the two processes from each other. NEB-juices located on the left of the plot correlated positively with sweetness due to the high sugar to acid ratio. NEB-juices correlated negatively with bitterness and astringency due to the high pectin content in the non-enzymatic juices, shown as the higher viscosity, which probably masked the astringency of the phenolic compounds. The EB-juices on the right side correlated with high bitterness and astringency due to the high content of phenolic compounds and lack of pectins in the juice. Phenolic compounds, especially hydroxycinnamic acids and flavonol glycosides, can be perceived as astringent already at low concentrations (Hufnagel & Hofmann 2008; Schwarz & Hofmann 2007). The former group contributed to puckering astringency, whereas the latter have more mouth-drying astringent properties (Hufnagel & Hofmann 2008). Various cultivars were distinguished in the

second factor, highlighting the strong sourness, astringency and bitterness properties of 'Breed 15'.

The process of EPR-juice pressing resulted in 9 times more phenolic compounds in the juices, than did the non-enzymatic pressing, and 5 times more than the conventional enzyme-aided berry-pressing (**Figure 13**) (**IV**). In the EPR-juice, the contents of monomeric anthocyanins, flavonols, and HCAs were 9-, 19-, and 11-fold, respectively, compared to the corresponding levels in the NEB-juice. The corresponding values were 5-, 3- and 3-fold, respectively, compared to the contents in the EB juices. The relative proportions of phenolic compounds were calculated for each juice process. Coumaric acid derivatives were the major HCAs (65–74%) consisting mostly of (*E*)-isomers (84–96%). Ferulic acid derivatives were the minor type of HCAs (13–14%). Nitriles of HCAs comprised the major (67–72%) type of derivatives, and *O*-glucosides the minor ones (2%) in each of the juice pressing processes.

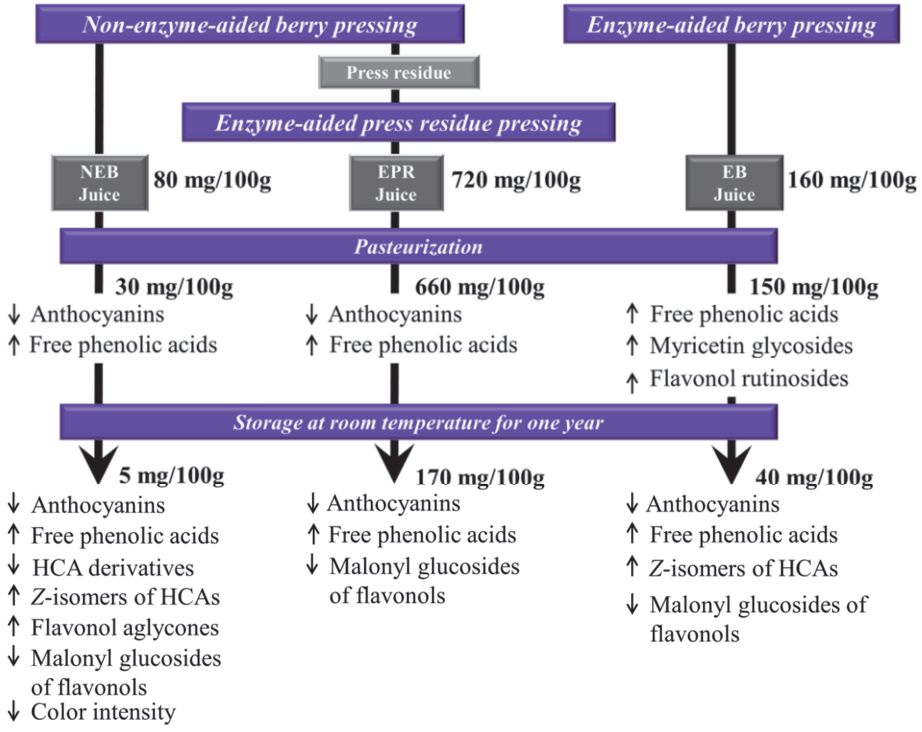

Figure 13. Summary of the impact of pasteurization and storage at room temperature on phenolic compounds of juices processed with different technologies (**IV**). The concentrations (mg/100g of juice) in bold are the sums of phenolic compounds, including anthocyanins, flavonols and HCAs.

For flavonol glycosides, myricetin was the major (58–68%) and isorhamnetin the minor aglycone (3%), with preferably glucose attached to the flavonol than rutinose. In anthocyanins, delphinidin and rutinose were the major aglycone and sugar moiety, respectively. The relative distribution of the aglycones and different types of derivatives of phenolic compounds were very similar among the three juices. Thus, the composition of phenolics in the pulp and in the skin were relatively similar, although the contents in the skin were notably higher.

The most significant change induced by pasteurization was the decrease of monomeric anthocyanins (**IV**) (**Figure 14A**). In the NEB-juice, the content of monomeric anthocyanins decreased down to 42% of the level before pasteurization. This was also visually observed as the lowered color intensity of the juice. In the EPR-juice, the decrease of monomeric anthocyanins was less (8%), but in EB-juice the compounds remained stable. In all the three juices, hydroxybenzoic acids and free HCAs increased during pasteurization. Flavonols stayed stable during processing. Surprisingly, pasteurization increased the content of myricetin glycosides and flavonol rutinosides in the EB-juice. It is likely that heating increased the release of these compounds, probably *via* hydrolysis of some polymers originating from the skin-fraction. In addition, the hydrolyzing activity of the native enzymes might have lasted until inactivation by the heat treatment.

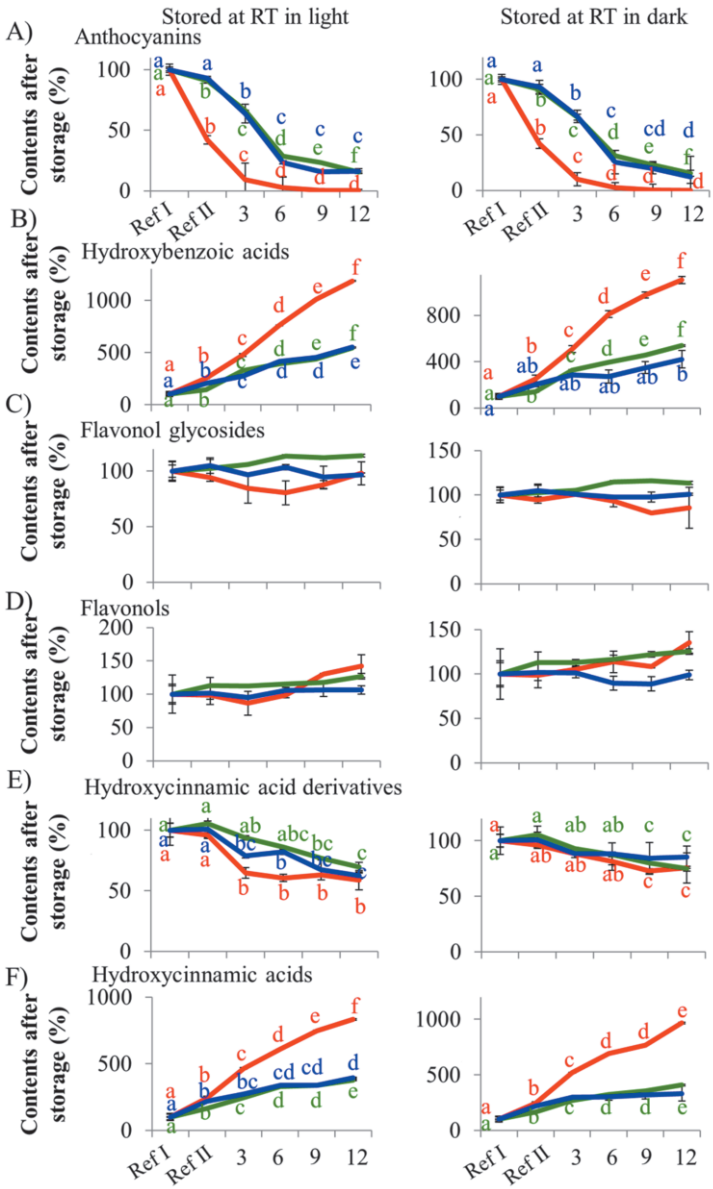

Figure 14. Changes in phenolic compounds during pasteurization and storage (**IV**). Degradation of (A) monomeric anthocyanins into (B) hydroxybenzoic acids; hydrolysis of (C) flavonol glycosides into (D) flavonol aglycones; hydrolysis of (E) hydroxycinnamic acid derivatives into (F) free hydroxycinnamic acids. NEB- ▬, EPR- ▬, and EB-juices ▬ during storage at room temperature (RT). Values before pasteurization (Reference I) are equal with 100% on y-axis. Different letters indicate statistically significant difference ($p < 0.05$) based on One-way ANOVA and Tukey's post-hoc test. The error bars are relative standard deviations (%).

5.3.2 Extrusion cooking

5.3.2.1 Extrusion process

The differences in the properties of the raw materials used in the production of extrudates presented challenges during drying, milling and extrusion cooking. The fresh non-enzymatic residue (NER) had a higher moisture content (moisture 80%) compared to the enzymatic residue (ER) (moisture 50%) because of the higher content of left-over juice in the former. A fairly low drying temperature of 40 ˚C was chosen in order to retain the original phenolic composition and other nutrients in the residue. Air-drying at 40 ˚C took twice as long for the NER residue than for the ER, resulting in some mold growth in some of the NER batches. Thus, NER residue ought to be dried with some other process technologies than bulk drying.

The bulk drying of NER resulted in large hard sticky pieces of the residue due to the high sugar content of the left-over juice, which made it difficult to mill the residue properly and to break down the seeds. Thus, the residue was crushed into coarse particles. The challenge with ER during milling was the released seed oil. With the addition of potato flour (7%), the dried ER was properly milled and the seeds were broken and passed through a sieve with a 0.5 mm screen.

ER-oat bran was extruded with the roughest process parameters to increase the expansion of the sample. Additionally, a high water feeding rate was used with the dough of ER-oat bran to prevent the extrudate being too dry because of the water binding properties of β-glucan in the oat bran (Yanniotis et al. 2007).

The NER based extrudates resulted in a greater expansion immediately after the die, due to their being less lipids in the residue. However, the high sugar content of NER resulted in the amorphous state of the melt after coming out of the die, and not being able to hold the expansion (Truong et al. 2004). The amorphism of the melt resulted in the bending of the snacks, and thus, the NER extrudates were cut earlier than the ER snacks.

5.3.2.2 Physico-chemical properties and consumer liking of the extrudates

Figure 15 summarizes the physical and chemical properties and the liking of the extrudates. A–C snacks (ER residue based snacks) and D–F snacks (NER residue based snacks) were compared with each other. Compared with ER-residue, the non-enzymatic residue (NER) extruded with barley or oat flour resulted in extrudates with a higher expansion, lower hardness and density, higher redness (a^*), lower pH, and higher contents of glucose, fructose and fruit acids, all contributing positively to a liking of the flavor, texture and

appearance, as well as berry-like experience by the consumers. These characteristics were obtained with more gentle processing parameters, consisting of a lower total mass flow, screw speed and barrel temperature, compared to the parameters used for the ER based extrudates. The higher redness of NER extrudates was most likely due to the higher content of anthocyanins in the NER, since non-enzyme-aided berry pressing left a higher content of anthocyanins in the press residue. Lower barrel temperatures during extrusion of NER retained the heat-labile anthocyanins to a higher extent compared to the extrusion of ER (Khanal et al. 2009a). Moreover, the lower pH of NER-based snacks, resulting from a higher fruit acid content, kept the anthocyanins in a more stable form of red flavylium cation (AH^+). The ER residue contributed to a higher density and hardness due to the higher content of lipids and fiber, all known to decrease expansion.

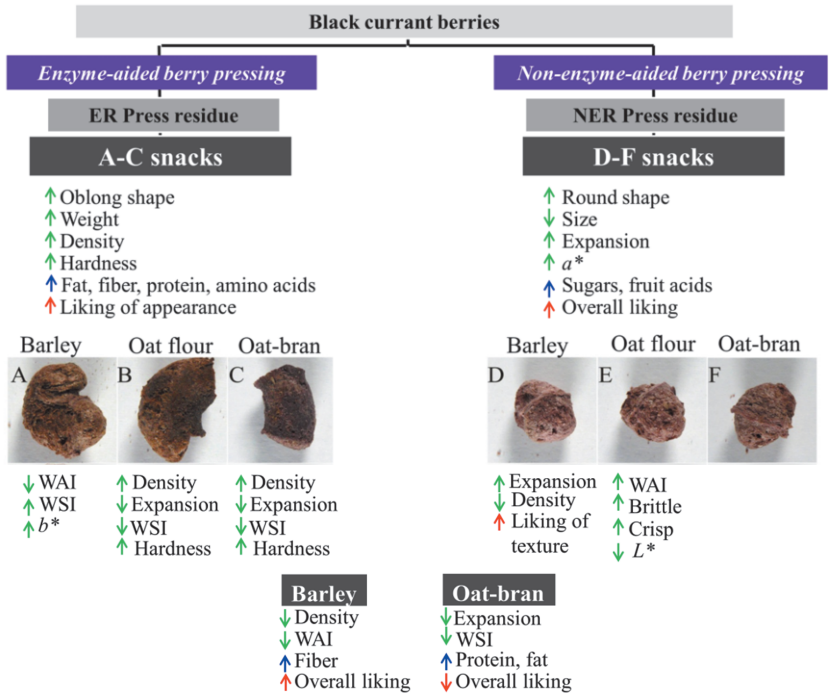

Figure 15. Summary of the physical (green arrows) and chemical (blue arrows) properties and hedonic response (linking, $n = 77$) (red arrows) of the extrudates (**II**). Arrows pointing upwards indicate increased property, downwards decreased property. Extrudates are produced of six different recipes: enzymatic residue (ER) extrudates of A) ER-barley, B) ER-oat and C) ER-oat bran; non-enzymatic residue (NER) extrudates of D) NER-barley, E) NER-oat and F) NER-oat bran.

5.4 Influence of storage

5.4.1 Influence of storage on individual hydroxycinnamic acids

The most significant change induced by storage among hydroxycinnamic acid compounds was the increase in the corresponding free HCAs (**Figure 16**). The increase was accompanied by a decrease or a minor increase in the corresponding derivatives (**Figure 17**) verified from NEB-juice (**III**). The contents of free (*E*)-caffeic and (*E*)-ferulic acids increased 3–4-fold at room temperature during the 12 months of storage, regardless of the light conditions. Free (*E*)- and (*Z*)-coumaric acids were released even to a higher extent. Light induced a drastic increase in the *Z*-isomer (**Figure 16A**).

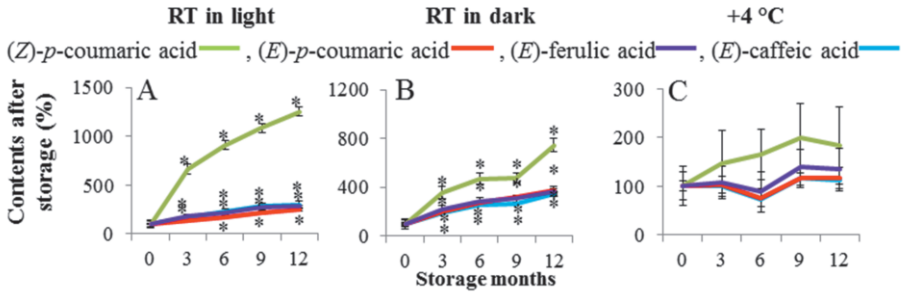

Figure 16. Changes in the content of free HCAs during storage of NEB-juice (**III**). Storage at A) room temperature (RT) in light, B) at RT in dark and C) at +4 °C. Values at 0-month (after pasteurization) of storage equal with 100% on y-axis. Statistically significant changes*, when compared with the baseline sample based on One-way ANOVA together with Tukey's post hoc and LSD test ($p < 0.05$). The error bars are relative standard deviations (%) between the replicates.

The increase in free HCAs was accompanied by a significant decrease in the contents of (*E*)-caffeoyl-, (*E*)-coumaroyl-, and (*E*)-feruloylglucoses, as well as (*E*)-coumaroyl- and (*E*)-feruloyloxymethyleneglucosyloxybutenenitriles at room temperature. The corresponding caffeoyl derivatives decreased to a lesser extent (**Figures 17A–F**). (*E*)-coumaroylglucose and (*E*)-coumaroyloxymethyleneglucosyloxybutenenitrile were converted into the corresponding *Z*-isomers by light storage. Storage at a cold temperature (+4 °C) kept the composition of phenolic acids rather constant with only minimal changes. The order of lability of HCA derivatives at room temperature was *O*-acylglucoses > acyloxymethyleneglucosyloxybutenenitriles > *O*-acylquinic

acids > *O*-glucosides. If the *O*-acyl bond in the derivatives of HCAs follows the hydrolysis pathway of esters, the most probable place for hydrolysis is the bond between carbonyl C-9′′ and oxygen (presented in the **Figure 8E** as a red dotted line), resulting in free hydroxycinnamic acids.

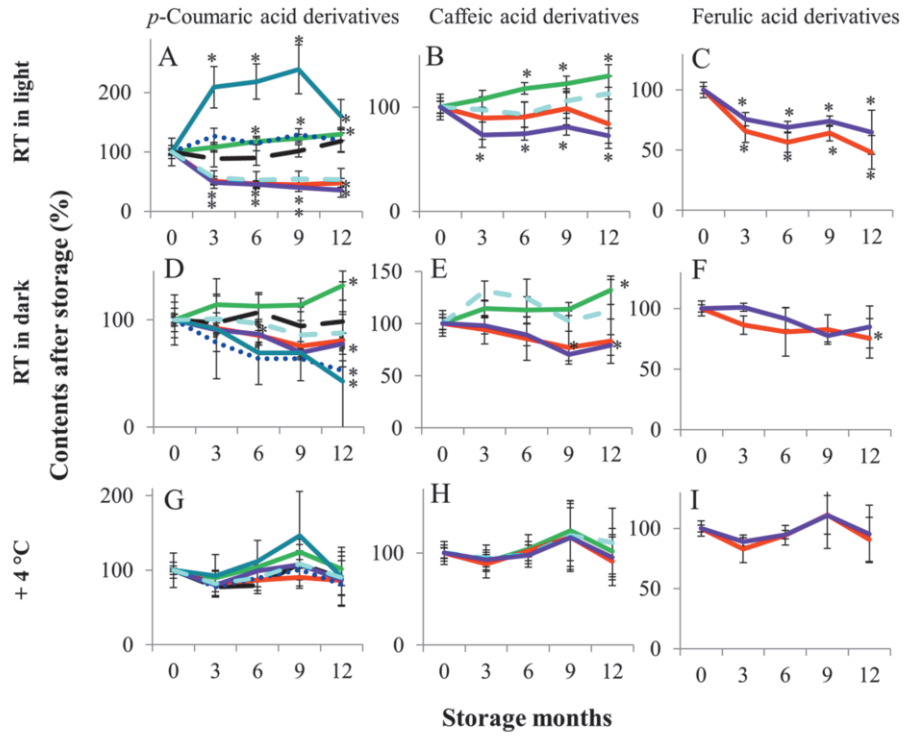

Figure 17. Decomposition of HCA derivatives in NEB-juice during one year of storage (**III**). (*E*)-caffeoyl / coumaroyl / feruloylglucose, (*E*)-coumaroyl-/caffeoyl-/feruloyloxymethyleneglucosyl-oxybutenenitrile, (*Z*)-coumaroyl-oxymethyleneglucosyloxybutenenitrile, neochlorogenic acid / (*E*)-*p*-coumaroylquinic acid, (*Z*)-coumaroylglucose, (*E*)-caffeic and (*E*)-*p*-coumaric acid *O*-glucoside, (*Z*)-*p*-coumaric acid *O*-glucoside. The co-eluting *O*-glucosides of (*E*)-caffeic and (*E*)-*p*-coumaric acids are represented by the same lines. RT, room temperature. Values at 0-month of storage equal 100% on y-axis. *Statistically significant difference compared with the baseline sample based on One-way ANOVA together with Tukey's post hoc and LSD test ($p < 0.05$). The error bars are relative standard deviations (%) between the replicates.

5.4.2 Influence of storage on phenolic compounds

The first PC in the PCA model (**Figure 12A**) showed that the type of juice processing had a more significant impact on chemical composition than storage. The second PC indicated that the original composition of phenolic compounds as sugar derivatives had remained the most stable during cold storage in the juices of the EPR process, located on the right side of the plot. The juices of NEB stored at room temperature, located opposite to the derivatives of phenolic compounds, indicates a decrease of these compounds in the juices. In the first factor of the PLS model (**Figure 12B**), the storage of NEB-juices correlated with sweetness. In the second factor, juices of different storage times did not vary significantly between one another.

Figures 13 and **14** summarize the influence of storage on the phenolic compounds of black currant juices stored at room temperature **(III**, **IV)**. The impact of cold storage (+4 °C) was also studied, but in cold storage many compounds mainly stayed stable (**III**, **IV**). However, during cold storage, monomeric anthocyanins decreased by 13–54%, the greatest loss occurring in NEB-juice and the smallest in EB-juice.

After one-year storage period, very similar changes were induced by light and dark storage at room temperature. At room temperature, only 17% of the total amount of phenolic compounds seen in the NEB-juice was left, and 26–27 % in the enzyme-aided juices, compared to the level after pasteurization (**Figure 13**). Again, the monomeric anthocyanins were the most vulnerable group of phenolic compounds (**Figure 14A**). Surprisingly, the decrease was independent of the exposure to light, as was the case also for every individual anthocyanin except delphinidin glycosides. Delphinidin glycosides showed greater loss in dark storage than light storage. Compared to the levels found immediately after pasteurization, only 0.7% of free anthocyanins in NEB-juice and 17% in the enzyme-aided juices were left after 12-months of storage at room temperature. The loss of the monomeric anthocyanins in the NEB-juice was also recognized visually as the lowered color intensity of the juice, turning from magenta into brownish. Similar changes in color were not observed in enzyme-aided juices due to the very high content of anthocyanins and intensity of the color.

Simultaneously with the decrease in anthocyanins, an increase of up to 2–5-fold in anthocyanin degradation products (hydroxybenzoic acids) was detected (**Figure 14B**). Free HCAs increased by a factor of two to four in all the juices (**Figure 14F**). The main difference between light and dark storage at room temperature was the light induced isomerization of (*E*)-coumaric acid derivatives into the corresponding (*Z*)-isomers in the NEB-and EB-juices, a phenomenom not seen in dark storage. Interestingly in the EPR-juice, the

highest concentrations of (*Z*)-isomers were already found in the baseline samples, with a decreasing trend during storage. Possibly the skin of the berry already contained fairly high amounts of (*Z*)-coumaric acid derivatives as a result of activated defence mechanisms towards sun- and UV-light radiation. The (*Z*)-isomers were extracted from the skin, in this case from the press residue, into the juice more abundantly than from the whole berry. The abundance of phenolic compounds in EPR-juice might also have protected the (*E*)-isomers from converting into (*Z*)-isomers during storage. Flavonols (**Figures 14C**, **D**) stayed rather stable during storage. However, the attachment of a malonyl-moiety to glucosides resulted in the lower stability of flavonol glucosides during storage.

6 CONCLUSIONS

The results of the practical work of the thesis showed that the selection of the raw material, processing technology and parameters as well as storage length and conditions, greatly contribute to the chemico-physical and sensory properties of black currant based products. The black currant cultivars contained varying contents of sugars and non-phenolic fruit acids, and phenolic compounds, which contribute to the sweetness/sourness, bitterness, and astringencies of the juices. The selection of the berry cultivar also affects the quality and consumer acceptance of the final products.

Juice processing technology had the more significant impact on the chemical and sensory characteristics of the juices, compared to cultivars and storage length and conditions. Non-enzymatic pressing of berries (NEB-juice) led to a higher yield of the pulp in the juice, having a high sugar to acid ratio and a higher sweetness, along with a high content of pectins masking the astringency and bitterness induced by phenolic compounds. The non-enzymatic pressing can be considered as an alternative pressing technology to produce more natural minimally processed juices, smoothies and purées with a more pleasant sensory quality. Due to the low juice yield, the berry pressing requires innovative exploitation techniques for the press residues.

Enzyme-aided juice pressing of berries (EB-juice) increased the yield of phenolics compared to non-enzymatic pressing. Enzyme treatment increased the intensities of bitterness and astringency, which may adversely affect the sensory quality and consumer acceptance of the juices. Enzymatic pressing of the non-enzymatic press residue (EPR-juice) extracted higher content of phenolic compounds from the skin of the berries, compared to the EB- and NEB processes. EPR-juice probably has stronger puckering and mouth-drying astringencies and bitterness due to the multiple times higher content of phenolic compounds. Both the enzyme-treated EPR- and EB-juices lacked the masking effect of pectins, further enhancing the astringency. The EPR-juice as such is probably too astringent for consumers. The juice could be used as a supplemental ingredient for increasing the phenolic content of fruit and berry products.

The high content of phenolic compounds in juices seemed to protect these compounds from decomposition caused by stress factors during processing and storage. EPR-juice, with the highest phenolic content, had a rather stable composition of phenolics during storage. The NEB-juice had the lowest content and the most labile composition of phenolic compounds, which was seen as the lowest stability of the juice color after one-year of storage. Of the 51 metabolites studied, anthocyanins and phenolic acids were the most labile

during thermal processing and storage at room temperature, the flavonols being the most stable group of phenolic compounds. At room temperature, the main difference between light and dark storage conditions was the light induced isomerization of (*E*)-coumaric acid and derivatives into the corresponding (*Z*)-isomers. Cold storage is necessary to preserve the original quality of the berry juices.

Extrusion cooking proved to be a viable method for exploitation of the press residues in various snacks and breakfast products. The berry-like characteristics, such as fresh berry taste and color, were well retained in the non-enzymatic press residue based extrudates. The higher fiber and lipid content of the conventional enzyme-aided press residue contributed to less favorable texture characteristics and as such lacked the unique flavor of berry materials. The berry-like flavor may be restored by adding some original juice or other berry fractions during the extrusion process. Extrusion cooking demonstrated new opportunities to exploit by-products from various berry-processing to meet the growing demand for healthy snacks and breakfast food products.

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DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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

39. **SAMPO LAHTINEN (2007)** New insights into the viability of probiotic bacteria.
40. **SASKA TUOMASJUKKA (2007)** Strategies for reducing postprandial triacylglycerolemia.
41. **HARRI MÄKIVUOKKO (2007)** Simulating the human colon microbiota: studies on polydextrose, lactose and cocoa mass.
42. **RENATA ADAMI (2007)** Micronization of pharmaceuticals and food ingredients using supercritical fluid techniques.
43. **TEEMU HALTTUNEN (2008)** Removal of cadmium, lead and arsenic from water by lactic acid bacteria.
44. **SUSANNA ROKKA (2008)** Bovine colostral antibodies and selected lactobacilli as means to control gastrointestinal infections.
45. **ANU LÄHTEENMÄKI-UUTELA (2009)** Foodstuffs and medicines as legal categories in the EU and China. Functional foods as a borderline case. (Law).
46. **TARJA SUOMALAINEN (2009)** Characterizing *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 as a new probiotic combination: basic properties of JS and pilot *in vivo* assessment of the combination.
47. **HEIDI LESKINEN (2010)** Positional distribution of fatty acids in plant triacylglycerols: contributing factors and chromatographic/mass spectrometric analysis.
48. **TERHI POHJANHEIMO (2010)** Sensory and non-sensory factors behind the liking and choice of healthy food products.
49. **RIIKKA JÄRVINEN (2010)** Cuticular and suberin polymers of edible plants – analysis by gas chromatographic-mass spectrometric and solid state spectroscopic methods.
50. **HENNA-MARIA LEHTONEN (2010)** Berry polyphenol absorption and the effect of northern berries on metabolism, ectopic fat accumulation, and associated diseases.
51. **PASI KANKAANPÄÄ (2010)** Interactions between polyunsaturated fatty acids and probiotics.
52. **PETRA LARMO (2011)** The health effects of sea buckthorn berries and oil.
53. **HENNA RÖYTIÖ (2011)** Identifying and characterizing new ingredients *in vitro* for prebiotic and synbiotic use.
54. **RITVA REPO-CARRASCO-VALENCIA (2011)** Andean indigenous food crops: nutritional value and bioactive compounds.
55. **OSKAR LAAKSONEN (2011)** Astringent food compounds and their interactions with taste properties.
56. **ŁUKASZ MARCIN GRZEŚKOWIAK (2012)** Gut microbiota in early infancy: effect of environment, diet and probiotics.
57. **PENGZHAN LIU (2012)** Composition of hawthorn (*Crataegus* spp.) fruits and leaves and emblic leafflower (*Phyllanthus emblica*) fruits.
58. **HEIKKI ARO (2012)** Fractionation of hen egg and oat lipids with supercritical fluids. Chemical and functional properties of fractions.
59. **SOILI ALANNE (2012)** An infant with food allergy and eczema in the family – the mental and economic burden of caring.
60. **MARKO TARVAINEN (2013)** Analysis of lipid oxidation during digestion by liquid chromatography-mass spectrometric and nuclear magnetic resonance spectroscopic techniques.
61. **JIE ZHENG (2013)** Sugars, acids and phenolic compounds in currants and sea buckthorn in relation to the effects of environmental factors.
62. **SARI MÄKINEN (2014)** Production, isolation and characterization of bioactive peptides with antihypertensive properties from potato and rapeseed proteins.
63. **MIKA KAIMAINEN (2014)** Stability of natural colorants of plant origin.
64. **LOTTA NYLUND (2015)** Early life intestinal microbiota in health and in atopic eczema.
65. **JAAKKO HIIDENHOVI (2015)** Isolation and characterization of ovomucin – a bioactive agent of egg white.
66. **HANNA-LEENA HIETARANTA-LUOMA (2016)** Promoting healthy lifestyles with personalized, *APOE* genotype based health information: The effects on psychological-, health behavioral and clinical factors.
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