DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

Novel Bioprocessing for Increasing Consumption of Nordic Berries

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ABSTRACT

Lingonberry is the highest yielding wild berry found in Finnish forests. Even though lingonberry is a commonly used berry in Finland, the annual harvesting rate is only approximately 10 % of the crop. Black currant is the second most cultivated berry crop in Finland. Both of the berries have a distinct attractive odour and intense flavour properties, which limits their use. Lingonberry and black currant are rich in phenolic compounds, such as anthocyanins, flavonols, and proanthocyanins, which have beneficial effects on human health. However, some of these compounds have negative effects on the berries sensory quality, causing lingonberry and black currant to have a bitter taste and an astringent mouthfeel. The aim of this research was to study the impact of bioprocessing and the application of biopolymers on the compounds contributing to the sensory properties of lingonberry and black currant, thus, investigating the possibility of modifying the flavour. A special focus was placed on the characterisation of the changes in the non-volatile and volatile flavour compounds. The goal was to produce new knowledge on the potential of bioprocessing to modify the quality factors of foods and provide guidance for the juice and fermentation industries as regards bioprocessing of Nordic berries.

Cyclodextrins are used to decrease negative sensory properties, such as bitterness and astringency, by forming inclusion complexes with compounds contributing to the sensory properties. Lingonberry juice diluted from a commercial concentrate was treated with a biopolymer, a cyclodextrin, gelatine, sequentially with two biopolymers, or with NaOH to increase the pH. Generally, the gelatine treatment decreased all the phenolic compound content and the cyclodextrin treatments slightly increased it. Treatment with cyclodextrins alone was not sufficient to modify the sensory properties of the lingonberry juice, but the sequential treatment with gelatine and cyclodextrin decreased the bitterness and astringency of the lingonberry juice.

Enzymatic treatments are commonly used in the juice industry to increase the juice yield. In addition, enzymatic treatments influence the chemical composition and, thus, the sensory quality of the final juice products. Two commercial pectinases, cellulase, and β-glucosidase and one research enzyme product were used to produce lingonberry juice from frozen Finnish lingonberries. Enzymatic treatments were conducted with the minimum or maximum enzyme dosage recommended by the producer and with incubation period of one- or three-hour at 50 °C. All the enzymes used significantly increased the juice yield and the content of most of the phenolic and volatile compounds. Incubation time had more effect on the contents of the phenolic

compounds by increasing the content of various phenolic compounds compared to the enzyme dosage.

Beverages with a low content of alcohol are becoming increasingly popular around the world. Non-*Saccharomyces* yeasts can be used in fermentation to produce soft alcoholic beverages with a low alcohol content due to their characteristic nature production a lower amount of ethanol. They may also have a positive effect on the sensory properties of alcoholic beverage. Black currant juice was prepared from frozen commercial Finnish black currants and fermented with *Saccharomyces cerevisiae*, *S. bayanus*, *Torulaspora delbrueckii*, and *Metschnikowia pulcherrima*, and *M. fructicola* yeasts without added sugar. The ethanol content, sugars, organic acids, certain phenolic compounds, and the volatile compounds were measured before and after fermentation. GC-O and generic descriptive sensory analysis was used to determine the sensory differences between the fermented beverages. The ethanol content varied significantly between the fermentations and the sequential fermentations with non-*Saccharomyces* and *Saccharomyces* yeasts resulted in the lowest ethanol levels. In general, all fermentations decreased the anthocyanin content and, thus, the colour properties of the fermented beverages. The content of glycosylated nitrile-containing hydroxycinnamic acids, a major phenolic compound group in the currants, were decreased after all the fermentations.

Volatile compounds have an important role in the flavour of fermented beverages. Oenological yeasts produce volatiles at different rates due to their metabolic differences and this results in the different sensory properties of the beverage produced. Ninety-eight volatile compounds were determined from the black currant juice and fermented beverages. All the fermentations significantly effected the volatile composition. Clear differences were observed in the volatile content between the used yeasts: Fermentation with *S. bayanus* resulted the highest content of esters and terpenes, where as *T. delbrueckii* ferementation had the lowest levels of these compound groups. The GC-O analysis also revealed clear difference between *S. bayanus* and *T. delbrueckii* fermented beverages. In the generic descriptive analysis, a significant difference was observed only in the ‘black currant odour’, the ‘musty odour’, and the viscous mouthfeel between the fermented beverages. Surprisingly, the *S. bayanus* fermented beverage had the highest ‘mustiness’ and lowest odour and flavour of ‘black currantness’, whereas these attributes were high in the *T. delbrueckii* fermented beverage.

The research of this doctoral thesis show that these selected bioprocessing methods have the potential to modify the chemical compositions and sensory properties of Finnish lingonberries and black currants. The research findings provide new insight and important references for the bioprocessing of these and other Nordic berries.

SUOMENKIELINEN ABSTRAKTI

Suomen metsissä villinä kasvavista marjoista puolukalla on suurin vuotuinen sato. Puolukkaa käytetään yleisesti suomalaisissa talouksissa, mutta silti sadosta kerätään vuosittain vain noin 10 %. Viljellyistä marjoista mustaherukka on toiseksi eniten viljelty marja Suomessa. Molemmilla marjoilla on houkutteleva tuoksu sekä voimakkaat makuominaisuudet, jotka vaikuttavat näiden marjojen käyttöön sellaisenaan. Puolukka ja mustaherukka sisältävät suuria määriä erilaisia fenolisia yhdisteitä, kuten antosyaaneja, flavonoleja ja proantosyanidiineja. Näillä yhdisteillä on mahdollisia terveyttä edistäviä ominaisuuksia. Samaiset haihtumattomat yhdisteet saattavat kuitenkin vaikuttaa kielteisesti marjojen aistittaviin ominaisuuksiin, koska niiden on kuvattu olevan maultaan karvaita ja suutuntumaltaan kurtistavia ja kuivattavia. Tämän väitöskirjatutkimuksen tavoitteena oli tutkia erilaisten bioprosessointien ja biopolymeerien vaikutuksia marjojen haihtuviin ja haihtumattomiin yhdisteisiin. Tutkimuksen tavoitteena oli tuottaa tieteellistä tietoa lupaavista bioprosessoinneista ja niiden käytöstä mehujen ja käymistuotteiden valmistuksessa suomalaisista marjoista.

Syklodekstriinit sulkevat yhdisteen sisäänsä, jolloin niitä voidaan käyttää vähentämään kielteisiä aistittavia ominaisuuksia, kuten karvasta makua sekä astringoivaa suutuntumaa. Kaupallisesta puolukkamehutiivisteestä laimennettua mehua käsiteltiin syklodekstriineillä, gelatiinilla, näiden polymeerien peräkkäisellä käsittelyllä tai natriumhydroksidilla pH:n nostamiseksi. Gelatiinikäsittely laski ja syklodekstriinit puolestaan nostivat puolukkamehun fenolisten yhdisteiden pitoisuuksia jonkin verran. Syklodekstriinikäsittelyt eivät yksinään muokanneet puolukkamehun aistittavia ominaisuuksia riittävästi, mutta peräkkäinen käsittely gelatiinilla ja syklodekstriinillä vähensi puolukkamehun karvasta makua ja astringoivaa suutuntumaa.

Entsyymikäsittelyjä käytetään mehujen valmistuksessa parantamaan saantoa. Ne vaikuttavat myös mehun kemialliseen koostumukseen ja siten myös aistittaviin ominaisuuksiin. Väitöskirjatyössä puolukkamehua valmistettiin käsittelemällä puolukoita kaupallisilla entsyymivalmisteilla (pektinaasi-, sellulaasi- ja *β*-glukosidaasientsyymit, kahtena pitoisuutena ja lämpökäsittelyajalla). Kaikki entsyymikäsittelyt nostivat merkittävästi mehusaantoa sekä fenolisten yhdisteiden pitoisuuksia. Pidempi lämpökäsittelyaika vaikutti entsyymin annostusta enemmän fenolisten yhdisteiden pitoisuuksiin.

Matalan alkoholipitoisuuden juomat ovat nouseva trendi ympäri maailmaa. Monet *Saccharomyces*-hiivoihin kuulumattomat hiivat tuottavat luonnollisesti vähemmän etanolia kuin *Saccharomyces cerevisiae*, minkä vuoksi niillä voidaan helposti valmistaa matalan alkoholipitoisuuden juomia. Näillä hiivoilla

on myös todettu olevan myönteisiä vaikutuksia alkoholijuomien aistittaviin ominaisuuksiin. Väitöskirjatyössä suomalaisista pakastemustaherukoista valmistettu mehu käytettiin ilman lisättyä sokeria *S. cerevisiae*, *S. bayanus*, *Torulaspora delbrueckii*, *Metschnikowia pulcherrima* ja *M. fructicola* viinihiivoilla. Etanolin, sokerien, happojen ja tiettyjen fenolisten ja haihtuvien yhdisteiden pitoisuudet määritettiin ennen ja jälkeen hiivakäymisen. Aistittavien ominaisuuksien muutoksia tutkittiin käyttäen kaasukromatografi-olfaktometria (GC-O) sekä yleistä kuvailevaa aistittavan arvioinnin menetelmää. Etanoli pitoisuus vaihteli merkittävästi eri viinihiivojen välillä ja alhaisin etanolipitoisuus oli *Metschnikowia* ja *Saccharomyces*-hiivoilla tehdyn peräkkäisen käymisen jälkeen. Kaikki hiivakäymiset alensivat antosyaani-väriyhdisteiden pitoisuuksia muuttaen siten juomien väriominaisuuksia. Tietyt mustaherukalle tyypilliset hydroksikanelihapot, jotka sisältävät typpeä ja glykosidiryhmän, ovat mustaherukan isoimpia fenolisten yhdisteiden ryhmiä. Näiden yhdisteiden pitoisuudet laskivat kaikkien käymisreaktioiden aikana.

Viinihiivat tuottavat eri määriä haihtuvia yhdisteitä niiden erilaisen aineenvaihdunnan vuoksi. Haihtuvilla yhdisteillä on tärkeä rooli käymistuotteiden flavorin muodostumisessa ja eri hiivoilla voidaankin saavuttaa hyvinkin erilaisia aistittavia ominaisuuksia. Väitöskirjatyössä mustaherukkamehusta ja -juomista havaittiin 98 haihtuvaa yhdistettä ja kaikki viinihiivakäymiset vaikuttivat merkittävästi juomien yhdistekoostumukseen. *S. bayanuksella* käyttäminen tuotti eniten estereitä ja terpeeneitä, kun taas *T. delbrueckii* käymisen jälkeen näitä yhdisteitä oli vähiten. Selkeä ero näiden hiivojen välillä oli myös havaittavissa GC-O-analyysissä. Yleisellä kuvailevalla menetelmällä merkittäviä eroja aistittavassa laadussa oli havaittavissa vain ”mustaherukan tuoksuisen”, ”tunkkaisen tuoksuisen” ja viskoosisen suutuntuman välillä. *S. bayanus* aiheutti tunkkaisimman tuoksun sekä vähemmän mustaherukkaisen tuoksun ja flavorin, kun taas nämä ominaisuudet olivat matalat *T. delbrueckii* käymisen jälkeen.

Tämän väitöskirjatutkimus osoitti valittujen bioprosessointimenetelmien mahdollistavan suomalaisen puolukan ja mustaherukan kemiallisen koostumuksen sekä aistittavien ominaisuuksien muokkaamisen. Tutkimuksen tulokset tarjoavat uutta näkemystä ja vertailukohtaa näiden ja muiden pohjoisten marjojen bioprosessointiin ja mahdollistaa marjojen paremman hyödyntämisen teollisuudessa.

LIST OF ABBREVIATIONS

(E)C	(epi)catechin,
(E)GC	gallocatechin
a*(+)	redness, (–) greenness
ANOVA	analysis of variance
arab	arabinoside
arab fur	arabinofuranoside
ATP	adenosine triphosphate
Aw	water activity
b*	(+) yellowness, (–) blueness
BA	benzoic acid
CA	p-coumaric acid
CD	cyclodextrin
CFA	caffeic acid
CHA	chlorogenic acid
CI	colour intensity
CT	colour tonality
Cy	cyanidin
DAD	diode array detector
der	derivative
DP	degree of polymerisation
DPPH	2,2-diphenyl-1-picrylhydrazyl
E	trans
EB	enzymatic treated beverage
EC	Enzyme Commission Number
EO	essential oil
EPG	endopolygalacturonase
ES	enzyme-substrate complex
ESI	electrospray ionisation
FA	ferulic acid
FB	fermented beverage
FG	flavonoid glycosides
FID	flame ionisation detector
FRAP	Ferric Reducing Ability of Plasma test
FRSA	free radical scavenging activity
G	gelatine
G2-β-CD	maltosyl-β-CD
G3P	glyceraldehyde 3-phospahe
gal	galactoside
GC	gas chromatograpahy

glc	glucoside
HA/HCA	hydroxycinnamic acid
HCA	hydroxycinnamic acids
HCDC	hydroxycinnamate decarboxylase
hex	hexoside
HILIC	hydrophilic interaction chromatography methodology
HMG	3-hydroxy-3-methylglutaroyl
HPP	high pressure processing
HP-β-CD	hydroxypropyl-β-CD
HS-SPME	headspace-solid phase microextraction
K	equilibrium constant
kcat	the number of the substrates converted by one active site per second
kcat/Km	the efficiency of an enzyme
Km	the Michaelis-Menten constant
L*	lightness; 0 = black, 100 = white
MF	*Metschnikowia fructicola*
MIC	minimum inhibitory concertation
MP	*Metschnikowia pulcherrima*
MS	mass spectrometry
MvG	malvidin glucoside
NADH	dihydronicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NEB	non-enzymatic treated beverage
NIF	nasal impact frequencie
NMR	nuclear magnetic resonance
O	olfactometry
o	orto
p	para
PA	procyanidin
PC	principal component
PCA	principal component analysis
PF	pure fermentation
PLS	partial least squares regression
PTFE	polytetrafluoroethylene
Q/Q	quadrupole-quadrupole mass spectrometry
Q-TOF	quadrupole time-of-flight mass spectrometry
que	quercetin
rham	rhamnoside
RI	retention index
RT	room temperature

SB	*Saccharomyces bayanus*
SC	*Saccharomyces cerevisiae*
SeqF	sequential fermentation with S. cerevisiae
SimF	Simultaneous fermentation with *S. cerevisiae*
SNIF	square of nasal impact frequencie
STD	standard compound
Tot.Ace	total acetates
Tot.Ald	total aldehydes
Tot.Est	total esters
Tot.Eth	total ethers
Tot.FA	total fatty acids
Tot.HA	total higher alcohols
Tot.Ket	total ketones
Tot.Ter	total terpenes
TA	titratable acidity
TAC	total antioxidant activity
TAnC	total anthocyanins
Td	*Torulaspora delbrueckii*
TF	total flavonoid content
TPC	total phenolic content
UHPLC	ultra-high-pressure liquid chromatography
UV	ultraviolet
VA	vanillic acid
Vmax	maximum velocity of the enzyme
xyl	xyloside
YAN	yeast assimilable nitrogen
Z	cis
α-CD	*α*-cyclodextrin
β-CD	*β*-cyclodextrin
γ-CD	*γ*-cyclodextrin
*Δ*E*	the total colour difference

LIST OF ORIGINAL PUBLICATIONS

I Kelanne, N.; Laaksonen, O.; Seppälä, T.; Yang, W.; Tuukkanen, K.; Loponen, J.; Yang, B. Impact of cyclodextrin treatment on composition and sensory properties of lingonberry (*Vaccinium vitis-idaea*) juice. *LWT - Food Science and Technology*. **2019** 113.

II Marsol-Vall, A.; Kelanne, N.; Nuutinen A.; Yang, B.; Laaksonen O. Influence of enzymatic treatment on the chemical composition of lingonberry (*Vaccinium vitis-idaea*) juice. *Food Chemistry*, **2021**, 339.

III Kelanne, N.; Yang, B.; Liljenbäck, L.; Laaksonen, O. Phenolic compound profiles in alcoholic black currant beverages produced by fermentation with *Saccharomyces* and non-*Saccharomyces* yeasts. *J. Agric. Food Chem.* **2021**, 68, 10128-10141.

IV Kelanne, N.; Siegmund, B.; Metz, T.; Yang, B.; Laaksonen, O. Volatile compounds and sensory profiles of alcoholic black currant (*Ribes nigrum*) beverages produced with *Saccharomyces* and non-*Saccharomyces* yeasts. *Submitted*.

1 INTRODUCTION

Lingonberry (*Vaccinium vitis-idaea*) is an evergreen shrub, which grows across Scandinavia, Europe, and North America. It is the highest yielding wild berry in Finland with an annual yield reaching 500 thousand tons of fresh berries. However, only 3 to 10 percent of the total annual lingonberry yield is harvested (Roininen and Mokkila, 2007). Black currant (*Ribes nigrum*) is a medium-size woody shrub growing in all the northern parts of world, especially in Europe and Asia. The black currant is the second most cultivated berry crop in Europe, as well as in Finland. In the 2018, Russia was the largest black currant producer with 398 thousand tons, whereas in Finland the crop was 1421 tons. Nonetheless, Poland is the primary exporting country and exports 80 to 90 % of its fresh black currants and black currant products. (FAOSTAT, 2020) Despite the high content of the bioactive compounds and the health promoting features, lingonberries and black currants are still poorly utilised in the food industry: this is mostly due to the challenging flavour characterised by the high intensities of the sourness, bitterness, and astringency (Laaksonen, Knaapila, Niva, Deegan, & Sandell, 2016). The profile and content of the non-volatile phenolic compound and sugar to acid ratio impact on the bitterness and astringency of berries (Laaksonen et al., 2014; Mäkilä, 2017; Viljanen et al., 2014). For example, strawberry and raspberry are described as sweet, fresh, soft, and not bitter and not astringent, whereas lingonberry and black currant are described as strong, sour, bitter, and astringent (Laaksonen et al., 2016). The flavonol contents of strawberries and raspberries (9.2 % and 41.2 %, respectively) are notably lower compared to the contents of lingonberries and black currants (Mikulic-Petkovsek et al., 2012b). Certain flavonols (Mäkilä, 2017; Sandell et al., 2009) and flavan-3-ols (Hufnagel and Hofmann, 2008) have especially been shown to impact on the astringent mouthfeel, whereas hydroxycinnamic acids (Laaksonen et al., 2010; Mäkilä, 2017) and their ethyl esters (Hufnagel and Hofmann, 2008) have an impact on both the astringency and bitterness. In addition, the concentration of phenolic compounds effects their taste properties: many phenolic acids are astringent in lower concentrations and bitter in higher (Hufnagel and Hofmann, 2008).

Lingonberries contain moderate levels of sugars, approx. 91 g/kg, of which 50 % is glucose, 46 % fructose, and 4 % sucrose. The main organic acid in the lingonberries is citric acid representing almost 90 % of all organic acids. The other organic acids are malic, fumaric, shikimic, quinic, and ascorbic acid. (Mikulic-Petkovsek et al., 2012a; Varo et al., 1984; Viljakainen et al., 2002; Viljakainen and Laakso, 2002; Viljanen et al., 2014; Vilkickyte et al., 2019) The sugar-to-acid ratio, which is a typical indicator of the sweetness or sourness in berries and fruits, is approximately 2.7 in lingonberries (Mikulic-

Petkovsek et al., 2012a). In addition, lingonberries contain high amounts of benzoic acid (0.6–1.3 g/kg) (Viljakainen et al., 2002; Viljakainen and Laakso, 2002; Viljanen et al., 2014; Visti et al., 2003), which is a natural antimicrobial preservative making the microbial fermentation of the lingonberries a very challenging task (Kalpana and Rajeswari, 2019). The insoluble dietary fibre and soluble dietary fibre content of fresh lingonberries are 6 g and 4 g per kg, respectively (Varo et al., 1984).

The content of sugars and organic acids in black currants are highly dependent on the cultivar and weather conditions with clear variations occuring between growing years (Woznicki et al., 2017; Zheng et al., 2009). On average, the total sugar content of black currants is 85 g/kg fresh weight, of which 41 % is glucose, 52 % fructose, and 7 % sucrose (Laaksonen et al., 2014; Suomela et al., 2012; Zheng et al., 2009). Citric acid is the main organic acid in black currants represetning 95 % of all organic acids. Other organic acids are malic, quinic, shikimic, and ascorbic acid. The sugar-to-acid ratio in black currants is approximately 2.4 (Zheng et al., 2009). The pectin content of the black currant is relatively high, 19 g/kg of berries (Varo et al., 1984), and the use of pectinase is common in black currant juicing processing.

The berries are generally rich in various bioactive compounds which have potential health benefits due to their anti-inflammatory, anti-antherothrombotic, hypoglycemic, and antioxidative properties (Kivimäki et al., 2012; Määttä-Riihinen et al., 2005; Törrönen et al., 2012). Many of the bioactive compounds, such as flavonoids, also affect the sensory quality of the berries. Anthocyanins play a key role in the colour, while phenolic acids, flavonols and procyanidins may have an influence on the bitter and astringent taste properties (Bujor et al., 2018; Hufnagel & Hofmann, 2008). The sugar-to-phenolic acids, sugar-to-flavonol glycosides, and organic acid-to-phenolic acids ratios can be used to indicate the total taste intensity, and the sour, bitter, and puckering astringency taste properties (Laaksonen et al., 2014). The primary anthocyanins in the lingonberries are galactoside, glucoside, and arabinoside of cyanidin. The total anthocyanin content of the lingonberries ranges between 27.7 and 59 mg/kg fresh weight. (Dróżdż et al., 2017; Kivimäki et al., 2012; Lee and Finn, 2012) The flavan-3-ol and procyanidin contents are relatively high in lingonberries. Hellström and Mattila (2008) reported the procyanidin content to be 1.6 g/kg fresh weight and Kylli et al. (2011) reported 12.3 g/kg dry weight; thus procyanidin accounted for 71 % of the phenolic contents they quantified.

Black currants are rich in phenolic compounds, affecting their colour and taste properties. Anthocyanins constitute up to 90–95 % of all polyphenolic compounds in black currants (Aneta et al., 2013; Laaksonen et al., 2013; Laaksonen et al., 2014), when the procyanidins are not taken account. The procyanidin fraction of polyphenols is approx. 20 %. The primary four

anthocyanins are galactosides and glucosides of delphinidin and cyanidin adding up to 99 % of total anthocyanin content. (Aneta et al., 2013) Sourness, bitterness and astringency are the typical sensory properties of black currants.

The odour of lingonberry is distinct, but there are only a few studies available describing the sensory properties of lingonberries (Anjou and von Sydow, 1967; Viljanen et al., 2014). However, the odour of black currant is well studied (Christensen and Pedersen, 2006; Jung et al., 2016; Liu et al., 2018; Marsol-Vall et al., 2018). In addition, the impact of processing on volatile compounds and the aroma of black currants has been investigated, such as that occurring during concentration (Piggott et al., 1993), nectar production (Iversen et al., 1998), and enzymatic treatment (Varming et al., 2004b). Sulphur-containing compounds, such as 4-methoxy-2-methyl-2-butanethiol, 2-isopropyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine, contribute to the 'catty' and 'musty' odours, which are characteristic to the black currants. (Jung et al., 2017, 2016)

In this thesis, cyclodextrins and gelatine, enzymatic treatments, and wine yeast fermentations were used to modify the chemical composition (non-volatile phenolic compounds or volatile compounds) and thus the sensory properties of lingonberries and black currants. In Study **I**, *β*- and *γ*-cyclodextrins were used as bitter blockers due to their inclusion complexation nature. The cyclodextrins were added to the non-treated commercial lingonberry juice or used sequentially with the gelatine treatment. Three pectinase, one cellulase, and one *β*-glucosidase products were used in the Study **II** to produce lingonberry juice on laboratory scale to affect the compounds contributing to the sensory properties. Study **III** and Study **IV** investigated the impact of different fermentation strategies, including single yeast fermentations and sequential fermentations with *Saccharomyces* and non-*Saccharomyces*, on the non-volatile (**III**) and the volatile chemical composition and sensory properties (**IV**) of the black currants.

2 REVIEW OF THE LITERATURE

2.1 Bioprocessing and biopolymers

Bioprocesses utilise microorganisms (e.g. bacteria, yeast, mould), their products (e.g. enzymes), or their parts (e.g. chloroplasts) to produce different products. Food bioprocessing is not new a phenomenon. It includes all procedures, which utilise living organisms, such as yeasts and different kind of acid bacteria, and enzymes. Figure 1 presents examples of the food bioprocessing using the biopolymers, fermentations, and enzymatic treatments. Fermentations, for example, are common food preservation procedures. Typical fermented foods are bread, yogurt, cheese, vinegar, and wine. The preserving effect is due to ethanol and acid production: when the acid or ethanol content increases, the growth of spoilage microorganisms is inhibited. Fermentation also changes the comprehensive chemical composition and the sensory properties of the raw material. For example, in yogurt fermentation, lactic acid bacteria use lactose to produce lactic acid. Lactic acid will eventually cause aggregation of casein, affecting the texture. The pH of the final yogurt will be significantly lower compared to milk; the texture and taste properties being different as well. (Zimmerman et al., 2020)

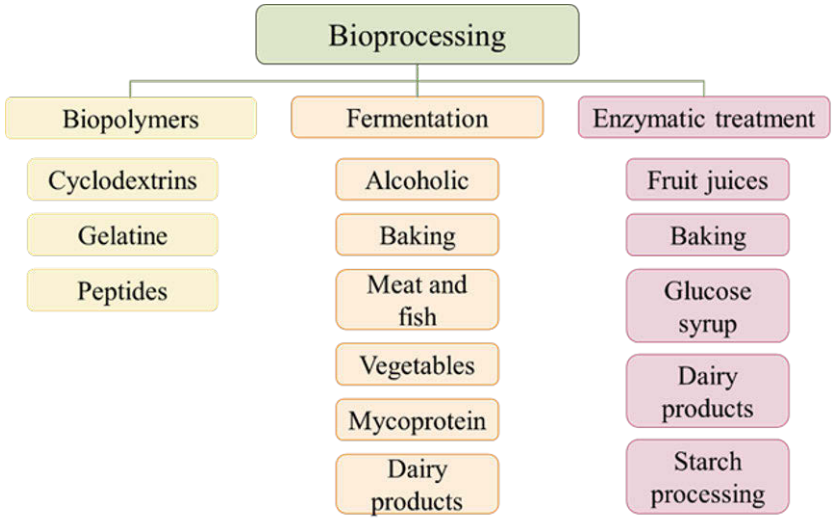

Figure 1. Examples of food bioprocessing (Grumezescu and Holban, 2018).

Biopolymers are diverse and versatile macromolecules produced by living cells or industrial environments. Biopolymers are composed from monomeric sugar, amino acid, or aliphatic polyester units. Different functional groups bind to the monomeric units and have an effect on the functionality of biopolymers. Two

of the major biopolymer classes are proteins and polysaccharides. Biopolymers play an important role in food processing due to their ability to improve physico-chemical properties and stability. (Grumezescu and Holban, 2018) Proteins are biopolymers, which are composed off the amino acid units. Collagen is the main structural protein in bone, skin, and tendon, making it the most abundant polypeptide and biopolymer. Gelatine is produced from collagen *via* partial hydrolysis. It is the most important protein produced from collagen. Gelatine is commonly used as an emulsifier, a stabiliser, a wine fining agent, and in the creation of biodegradable films. Gelatine is positively charged making it capable of binding negatively charged compounds, such as flavonoids. However, proanthocyanidins and tannins are bind by gelatine *via* hydrogen-bonding. This interaction is stronger between gelatine and high molecular weight compounds, such as proanthocyanidins with higher degree of polymerisation, compared to dimeric proanthocyanidins. (Waterhouse et al., 2016)

Polysaccharides, such as cellulose, starch, and pectin, are composed from monosaccharide units. Starch is used as raw material in cyclodextrin production, where cyclodextrin glycosyl tranferases (CGTase, EC 2.4.1.19) and α-amylases are used to hydrolyse starch at the α-(1-4)-position and modify the length of non-cyclic dextrin. After hydrolysation, intramolecular cyclisation leads to the formation of the cyclodextrin. The size of formed cyclodextrins formed depends on the substrate, the origin of the CGTase, and the reaction conditions. (Biwer et al., 2002)

Biopolymers can be used in the microencapsulation of a wide variety of food compounds, such as polyphenols, lipids, and volatile compounds. The encapsulation of compounds can improve the stability and bioavailability of the compounds and reduce unpleasant taste properties. However, encapsulation can also cause undesirable changes in the food matrix, such as a decrease in antioxidant activity. (Gómez-Mascaraque et al., 2018)

2.2 Cyclodextrins in food products

Cyclodextrins (CDs) are tapered cyclic oligosaccharides composed of six (α-CD), seven (β-CD), or eight (γ-CD) α-1,4-linked α-D-glucopyranoside units (**Figure 2**). The CDs are produced from starch by a relatively simple enzymatic conversion. The cavity sizes of the CDs increases by the number glucopyranoside units bearing the size of 0.56 nm, 0.70 nm, and 0.88 nm, respectively. (López-Nicolás et al., 2014) Since the glucopyranoside units are α-1,4-linked, the secondary hydroxy groups on the C-2 and C-3 are on the wide edge of the CD cone, and primary hydroxy groups of C-6 are on the narrow edge. The hydrogens, H-1, H-2, and H-4, of CH groups are located on the outer

part of CD molecule and the polar hydroxy groups oriented to the inner phase, resulting in the outer surface of the CD cone becoming hydrophilic and the inner surface hydrophobic. These structure features of CD provide the aqueous solubility and enables formation of the inclusion complex with the hydrophobic molecule (guests) or the hydrophobic part of compound in the aqueous environment. The guest molecule can be in a solid, liquid, or gaseous state. The inclusion complex does not break or form any covalent bonds. In an aqueous solution, the CD cavity is occupied by water molecules, which is readily substituted by more hydrophobic guest compounds. Due to the hydrophobicity of the cavity, the hydrophobic compound is more energetically favourable for the apolar-apolar association. Other binding forces, such as van der Waals interaction, hydrogen bonding, hydrophobic interactions, changes in solvent-surface tension, and release of ring strain in the CD molecule, may have importance in the binding of the guest molecule (Del Valle, 2004; Marques, 2010; Szejtli, 1998a).

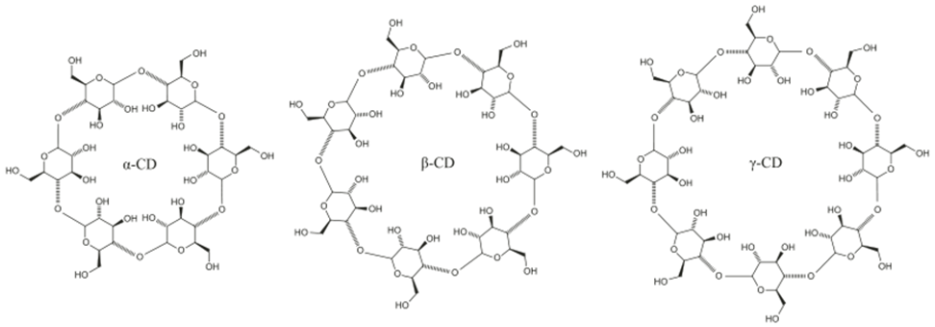

Figure 2. Two-dimensional illustration of *α*-, *β*-, and *γ*-cyclodextrins (Skowron, 2021).

In the inclusion complex, one molecule is enclosed within another molecule or an aggregation of molecules (Marques, 2010). Encapsulation of the guest molecule is not fixed or permanent, but a more dynamic equilibrium. Inclusion complex kinetics between the CD and the guest molecule depends on the relative size, shape, and hydrophobicity rate of the guest to the CD. (Del Valle, 2004; Szejtli, 1998b) Moreover, smaller molecules have greater complexing activity with CD and larger compounds are more dependent on the suitable functional group or ring to enter and interact with the CD cavity (Marques, 2010). Casado-Vela et al. (2006) studied the chelation of chlorogenic acid, epicatechin, protocatechuic acid, dopamine, L-3,4-dihydroxyphenylalanine, pyrocatechol, 4-tert-butylcatechol, and 4-methyl catechol with the methyl-*β*-CD. They concluded that the epicatechin, chlorogenic acid, and caffeic acid are more readily chelated by the methyl-*β*-CD than the dopamine, protocatechin, and 4-methyl catechol at low concentrations of methyl-*β*-CD.

Stability of the inclusion complex is also an important factor in the use of CDs. The water-solubility of the guest molecule effects the stability of the formed complex with the CD: more water-soluble molecules, such as sugars and organic acids, forms a less stabile complex compared to hydrophobic compounds (Zhang et al., 2012). Higuchi & Connors (1965) have established the phase-solubility diagrams, which shows how CDs and guest molecules can interact in the solution (**Figure 3**). Diagrams can be used to establish the stoichiometry of formed inclusion complex and derive the equilibrium constant (K) with the equation 1, if stoichiometry is known or is assumed to be 1:1 (CD:guest). Using a phase-solubility diagram, guests and CD complexation can be examined. The guest molecule is added in excess amounts into the water. The CD is introduced into the liquid system, which is then mixed at a constant temperature, until the complexation equilibrium is established. Solid guest molecules are removed and the concentration of the removed part is determined. Then the complexed guest content can be calculated. The CD concentrations (**Figure 3**, x-axis) and complexed guest (y-axis) contents are plotted in the diagram. In the phase-solubility diagram, an A-type system indicates the formation of the soluble inclusion complexes and a B-type system indicates the formation of poorly soluble or insoluble complexes. (Del Valle, 2004)

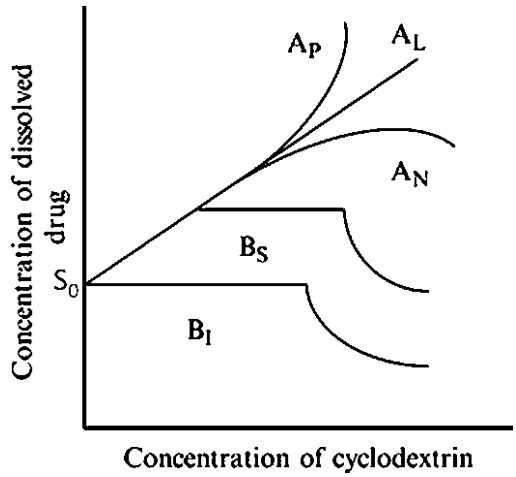

Figure 3. Phase-solubility diagrams by Higuchi and Connors (1965). S_0 solubility of guest without solubiliser; A_L linear increase of drug solubility as a function of CD concentration; A_P positively deviating isotherms; A_N negatively deviating isotherms; B_S complex with poor solubility; B_I complex insoluble.

$$K_{1:1} = \frac{[complex]}{[CD][guest]} = \frac{slope}{S_0(1 - slope)} \quad (1)$$

Where K1:1 is equilibrium constant, when CD:guest stoichiometry is 1:1, the slope is obtained from the phase-solubility diagram (slope have to be linear, i.e. AL-type), and S_0 is the intrinsic solubility of the guest molecule.

Several studies have shown that CDs form 1:1 stoichiometry complexes with flavonoids and volatile aroma compounds (Alvarez-Parrilla et al., 2005; Decock et al., 2006; Decock et al., 2008; Fernandes et al., 2014; Yang et al., 2011; Zhang et al., 2008, 2012), but other ratios, such as 2:1 and 1:2 (CD:guest), are also possible. The complexation ratio depends on the structural features of the molecules such as the length of the carbon chains and the types of functional groups (Cheirsilp and Rakmai, 2017). The formation of an inclusion complex can be determined by, for example, with the oxidative differential scanning calorimetry (DSC; Andrade et al., 2017; Hill et al., 2013), the Fourier transform infrared spectroscopy, powder X-ray diffractogram, ESI-MS (Zhu et al., 2019), and solid state ^{13}C NMR (Marques et al., 2019). However, different methods can often product a different stability constant for the same compound (Ciobanu et al., 2013). For example, Ciobanu et al. reported a stability constant of 105 M^{-1} between menthol and γ-CD determined with the stabile-headspace GC, whereas Layre et al. (2002) did not find any inclusion complexation between the menthol and γ-CD, when determining the HPLC using phase-solubility diagrams for the calculations.

The stability of the inclusion complex can be controlled by changing the environmental conditions, such as the CD or substrate concentration, the solution temperature, the pH, and the polarity of the solvent, or by the addition of a competitive compound (Marques, 2010). Different CDs can form more stable inclusion complexes with different-sized guests. α-CD forms typically stable inclusion complexes with the low molecular weight molecules or compounds with aliphatic chain, β-CD with heterocyclic compounds and aromatic rings, and γ-CD with larger compounds, such as steroids or macrocycles (Astray et al., 2009; Szejtli, 1998a).

Inclusion complexation between a poorly soluble guest compound and a CD has some consequences. When the guest is dissolved to the aqueous solvent, its concertation increases significantly, and the concentration of the CD decreases significantly. In the case of ionized guests or phenolic compounds, dissolvation of the compounds may enhance the solubility of the CD. Encapsulation with the CD changes the spectral properties of the guest: maximum of UV spectra may be shifted by several nanometers and fluorescence is strongly improved. In addition, achiral compounds become optically active in the chiral CD cavity and chemical shift of the anisotropically shielded atoms are modified in the NMR spectra. The interaction with the CD cavity also decreases the reactivity, diffusion, and the volatility of guest compound. Furthermore, the CD can act as

an artificial enzyme, accelerating various reactions, and modifying reaction pathways. (Szejtli, 1998a)

α-, *β*-, and *γ*-CD are the so-called native cyclodextrins, because they can be produced from starch by enzymatic reaction. Although native CDs and their complexes are hydrophilic, their solubility in aqueous solutions is usage-limiting characteristic, especially for *β*-CD which has the lowest water-solubility. To overcome this limitation, the CD derivatives are produced by chemical modification. The derivation of the CD changes the water solubility, extends the cavity size and, thus, increases the van der Waal interactions with the guest compound, increases stability against light and oxygen, and reduces effective cavity polarity. The chemical modifications are usually produced by the etherification, esterification, aminations, or tosylation of the primary and secondary hydroxyl groups of the cyclodextrins. Examples of the CD derivatives are hydroxypropyl-*β*-cyclodextrin (HP-*β*-CD), maltosyl-*β*-cyclodextrin, randomly methylated *α*-, *β*-, *γ*-cyclodextrins, and thiol-*β*-cyclodextrin (Decock et al., 2008; Del Valle, 2004; Manta et al., 2013; Wagner, 2006). Many studies have shown the positive effect of the modified CDs compared to the native CDs (Kulcan et al., 2019; Lucas-Abellán et al., 2019; Manta et al., 2013; Martínez-Hernández et al., 2019; Sojo et al., 1999; Zhang et al., 2008). However, the use of cyclodextrin derivatives in the food industry is limited by their possible toxicity and high costs; therefore only a few of them are produced at an industrial level.

Eventhough CDs usually contains 6–8 *α*-D-glucopyranoside units; they can also be constructed with a higher number of *α*-D-glucopyranoside units. Cyclodextrins containing as high amount as 60 glucopyronoside units have been reported (Takaha and Smith, 1999). These large-ring cyclodextrins (LR-CDs) can be enzymatically formed with cyclodextrin glucanotransferases (CGTases), for example, from tapioca or rice starch (Cao et al., 2020b; Kuttiyawong et al., 2015). LR-CDs have a larger hydrophobic inner cavity, which can interact even with the macromolecules, but also with smaller molecules, such as volatiles. LR-CDs can form an inclusion complex with both organic and inorganic molecules. The water solubility of LR-CDs is greater than *β*-CD and they have a flexible structure. Due to this flexible structure, the CDs of more than 26 structure units fold into an 8-shape forming two hydrophobic cavities (Cao et al., 2020a; Takaha and Smith, 1999; Zheng et al., 2002). LR-CDs can used to encapsulate, for example, *α*-tocopherol (Cao et al., 2020b) and essential oils (Cao et al., 2020a).

2.2.1 Application of cyclodextrins in food processing

The CDs may potentially be used to micro-encapsulate various compounds in food products with the aim of: I) protection of oxygen, light, or temperature

sensitive compounds from degradation; II) stabilisation of vitamins, fragrances, flavours, and essential oils; III) improved solubilisation of food colour and flavour agents and vitamins; IV) controlled release of certain food constituents; V) suppression of unpleasant tastes and odours, such as bitterness and off-flavours; and VI) removal of cholesterol or fatty acids (Andreu-Sevilla et al., 2011; Astray et al., 2009; Del Valle, 2004; Deshaware et al., 2018; Howard et al., 2013; Konno et al., 1982; Marques, 2010; Nedovic et al., 2011; Szente and Szejtli, 2004). In addition, CDs are used in the green and sustainable extraction of the compounds, such as phenolic compounds, from different parts of plants (Cui et al., 2017; Korompokis et al., 2017; Ratnasooriya and Rupasinghe, 2012). Examples of the effects of cyclodextrins on the chemical and sensory properties of food products are presented in the Table 1 and discussed in the following chapters.

Table 1. Effects of the cyclodextrins on the chemical and sensory properties of food products.

Publication	Raw material	Cyclodextrins	Methodology	Analysed parameters	Effects on compounds important for sensory properties
Konno et al. (1981)	Orange Grapefruit Amanatsu	*β*-CD 0.3 % (w/w)	CD and 8 % (w/w) of sucrose were added to the juice, juice heated to 95 °C for 10 min, cooled to RT. Sensory evaluation with trained panel	Bitterness	Addition of CD decreased bitterness significantly of all studied citrus fruits.
Shaw et al. (1984)	Grapefruit Navel orange	*β*-CD, 1 g/L of CD/50 mL of juice	CD in the continuous flow fluid-bed or in batch process, sensory evaluated with trained panel.	Concentration changes of limonin, nomilin, and naringin and impacts on the sensory properties	Cyclodextrin polymer treatment decreased approximately 50 % of bitter composition and sensory panel preferred debittered juices over non-treated.
Mongkolkul et al. (2006)	Tangerine	*β*-CD (1/3/5 g%)	Batch process, packed bed column process, and fluidised bed process with varying CD concentrations and process parameters were used.	Limonin content	*Batch processing*: direct correlation between CD content and limonin complexation. *Column processing*: 3 % CD had better debittering result (94 % limonin reduction) than batch processing. *Fluidised bed process*: juice flow rate effected on the binding rate of limonin, CD content did have only little effect on limonin reduction.
López-Nicolás et al., (2007)	Apple	G_2-*β*-CD (0/30/60/90 mM)	Selected concentration of CD in the 25 mL of distilled water.	The CIE coordinates, lightness (L*), red-green (a*), yellow-blue (b*), and the total colour difference (ΔE*)	Increased level of G_2-*β*-CD decreased to a large extent the total colour difference and slowed down the changes of L*, a*, and b*.

Publication	Raw material	Cyclodextrins	Methodology	Analysed parameters	Effects on compounds important for sensory properties
López-Nicolás and García-Carmona (2007)	Peach	α-CD (0/10/30/60 mM) G_2-β-CD (0/10/20/30 mM β-CD (0/3/5/10 mM)	Selected concentration of CD in the 25 mL of distilled water.	The CIE coordinates, lightness (L*), red-green (a*), yellow-blue (b*), the total colour difference (ΔE*), hue (H*), and chroma (C*)	Increased level of α-CD slowed down the changes of L* and ΔE*, and 60 mM of α-CD eliminated the change of L*. β-CD did not have effect on the colour of the peach juice in any concentration. increased level of G_2-β-CD slowed down the changes of L* and ΔE*. A- and G_2-β-CD had similar effects on the colour of peach juice.
Mourtzinos et al. (2008)	Roselle extract	β-CD at level of 1:1 anthocyanin concentration in extract	Extract heated to 60, 70, 80, and 90 °C for 10-110 min.	TAnC	Increased heat and time increased anthocyanin degradation, but β-CD decreased it. β-CD nearly doubled the half-time values of anthocyanins.
López-Nicolás et al. (2009)	Pear	α-CD (0/15/45/90 mM)	Freshly pressed pear juice was mixed with distilled water containing CD. All juice samples were oxidised at stirrer for 20 min.	The CIE coordinates, lightness (L*), red-green (a*), yellow-blue (b*), and the total colour difference (ΔE*), volatile composition, and sensory evaluation with trained panel.	Increased CD content delayed colour changes. Oxidation increased concentrations of certain volatile compounds. Use of CD decreased contents of volatiles in concentration dependent manner. Only high CD content significantly modified volatile profile. 15 mM of CD had a significant positive effect on the sensory quality and 90 mM led to the deterioration of aroma and odour attributes.
Andreu-Sevilla et al. (2011)	Pear	α-, β-, γ-CDs (15 mM)		Enzymatic browning, volatile compounds, colour, sensory properties	CDs slowed enzymatic browning. Bigger cavity size produced more efficient complexation with volatile compounds and changed the volatile profile. γ-CD significantly decreased the aroma and odour intensities, β-CD provided best colour, and α-CD resulted increased global quality of pear juice.

Publication	Raw material	Cyclodextrins	Methodology	Analysed parameters	Effects on compounds important for sensory properties
Navarro et al. (2011)	Mandarin juice enriched with pomegranate and goji berries	*β*-CD and HP-*β*-CD	Mandarin juice (mandarin 96 %, goji berries 2 %, pomegranate extract 1 %, CD 1 %) was pasteurised (98 °C 30 s) and stored for 75 d at 4 °C.	Stability of vitamin C, colour, and retinol equivalent, antioxidant capacity and sensory properties after storage	Control juice had the most intense fresh mandarin aroma, *β*-CD had the second highest and HP-*β*-CD lowest. HP-*β*-CD had the best overall quality, the highest value of colour intensity, vitamin C content, and retinol equivalents.
Howard et al. (2013)	Chokeberry	*β*-CD (0/0.5/1/3 %) in pH levels (2.8/3.2/3.6)	Different level of BCD in different pH levelled chokeberry juice. Storage at 25 °C and 4 °C for 2, 4, 6, and 8 mon.	Monomeric anthocyanins	Both pH and *β*-CD content effected on the stability of anthocyanins during storage. Ambient storage temperature effected more on the degradation of anthocyanins than refrigerator temperature.
Manta et al. (2013)	Apple	*β*-CD thiol-*β*-CD	Apple slices were treated with 700 µM (in 0.1 M sodium acetate buffer, pH 4.6) of *β*-CD or thiol-CD, incubated ate room temperature for 24 h.	Inhibition of the enzymatic browning (CIE)	Thiol-CD exhibited considerably higher inhibition of the enzymatic browning. Thiol-CD treatment resulted lighter, less red, and yellow colour of apple slices, indicating less browning.
Deshaware et al. (2018)	Bitter gourd	*β*-CD (0.25-2 %)	β-CD was added to freshly pressed juice and constatly stirred for 1 h at 25 °C, pH was adjusted to 3.5 and 2 g/L of stevia was added. Juice was pasteurised at 95 °C for 2 min.	Effect on sensory quality, TPC, TAC, and antidiabetic potential	All studied CD concentrations decreased bitterness. Addition of 1.5 % of CD resulted the most acceptable juice. Increased level of CD increased TPC and TAC. Marginal reduction in antidiabetic activity was observed.
Kulcan et al. (2019)	Pomegranate	*β*-CD, HP-*β*-CD (0.5/1/2 %)	Pomegranate juice treated with different level CDs and stored 3 months at 25 °C.	Colour (CIE), monomeric and polymeric anthocyanins, TPC, FRSA	CD type and level effected on the degradation rate of anthocyanins. HP-CD stabilised more anthocyanins than *β*-CD. *β*-CD did not show protective effect on the TPC. 0.5 % of HP-*β*-CD significantly increased TPC.

Publication	Raw material	Cyclodextrins	Methodology	Analysed parameters	Effects on compounds important for sensory properties
Martínez-Hernández et al. (2019)	Apple	*α*- (10/30/40 mM) and *β*-CD (5/10/15 mM)	Apple juice with addition of CD is treated with HPP (0/300/400/500 Mpa; 5 min, 22 °C).	Browning index and phenolic compounds	*α*-CD at 30 mM and *β*-CD at 15 mM level reduced the most HPP induced browning.

CD cyclodextrin; FRSA free radical scavenging activity; HP hydroxy propyl; HPP high pressure processing; G_2-*β*-CD maltosyl-*β*-cyclodextrin; TPC total phenolic content; TAC total antioxidant capacity; RT room temperature; TAnC total anthocyanin content; The CIE coordinates: L* lightness, a* red-green, b* yellow-blue, ΔE* the total colour difference

2.2.2 Effects of cyclodextrins on colour preperties

The colour is the first thing a consumer perceives about the food products making the appearance of food and beverages one of the most important characteristics. Anthocyanins are natural blue, red, and purple pigments. Anthocyanins are labile compounds affected by several environmental factors, such as pH, temperature, light, oxygen, co-pigment formation, enzymes, metal ions, and antioxidants (Howard et al., 2013; Khoo et al., 2017; Mäkilä et al., 2016; Rubinskiene et al., 2005). Howard et al. (2013) studied the effects of pH, *β*-CD, and storage temperature on the anthocyanins of chokeberries (**Table 1**). Their results showed that pH, *β*-CD concentration, and pH×*β*-CD interaction positively effected on the anthocyanin contents compared to the juices that were not treated. They concluded that the juice containing 3 % of the *β*-CD at natural pH of chokeberry (3.6) had 49 % more anthocyanins than the juice without *β*-CD addition after 8-month storage. Mourtzinos et al. (2008) studied the thermal stability of the anthocyanins of the roselle extract with and without the *β*-CD (**Table 1**). They observed higher thermal degradation with higher temperatures and longer treatment time, but the presence of the *β*-CD decreased the degradation rate and nearly doubled the half-life of the anthocyanins. In addition, Fernandes et al. (2018) reported increased thermal stability of the blackberry anthocyanins and decreased degradation of the anthocyanins under simulated gastrointestinal conditions when *β*-CD was used. Encapsulation of the anthocyanins in the CDs may increase the retention during food processing and the shelf life, but it can also fade the anthocyanin colour (Fernandes et al., 2013). Fading is also known as anti-copigmentation phenomenon. It occurs when the colourless forms of anthocyanins, quinoidal base, hemiketal, and chalcone, are more preferred in the inclusion complexation. This leads to a shift in the pigment hydration equilibrium towards the formation of more colourless forms of anthocyanins. Environmental pH, concentration of the *β*-CD and anthocyanin structure has an effect on the rate of anti-copigmentation. (Fernandes et al., 2013)

Browning of fruits and vegetables during the processing effects their appearance, taste properties, and nutritional values. The browning is typically caused by the polyphenol oxidases (PPOs), which catalyse the oxidation of mono- and *o*-diphenols to their corresponding quinones, which are polymerised with protein or amino acids resulting in high molecular weight structures called melanin or melanoidin (Gacche et al., 2003). For tropical and subtropical fruits, the browning causes as high as 50 % of the losses (Queiroz et al., 2008). CDs can be used to slow down the enzymatic browning after juice processing by binding the enzyme substrates (Casado-Vela et al., 2006; Irwin et al., 1994; José M. López-Nicolás et al., 2007a; López-Nicolás et al., 2009) or they may act as secondary antioxidants by preventing premature oxidation of the primary

antioxidant, such as an ascorbic acid (José M. López-Nicolás et al., 2007a). The effectiveness of the CDs to inhibit the enzymatic browning is highly dependent on the enzyme substrates and, furthermore, the stability constant between the CD and substrates. The higher the stability constant, the better the inhibition activity is. (Alvarez-Parrilla et al., 2007; Rosa et al., 2010) The studies about the impact of CD on the browning of banana pulp resulted in observations contradictory to the findings in other fruit juices, and the use of CDs was even found to activate browning in crude banana extracts (José M. López-Nicolás et al., 2007b; Sojo et al., 1999). Both these banana studies concluded that this phenomenon was caused by CD complexation of the natural browning inhibiting substances. Furthermore, Ghidelli et al. (2013) reported that at CD concentration of 10–50 mM did not significantly affect the browning of persimmon extract or precipitate compared to the control sample.

In addition to their inhibition effects on browning, CDs also have an effect on the rate of browning. Andreu-Sevilla et al. (2011) observed that the effect of three native CDs on the different rates of colour changes in pear juice (**Table 1**): *α*-CD slowed down the browning the most and *γ*-CD the least. On the other hand, López-Nicolás, Núñez-Delicado, et al. (2007) observed that maltosyl-*β*-CD and *β*-CD reduced browning more than *α*-CD when they studied apple juice (**Table 1**). CDs are reported to be effective against browning when they are used together with other polymers. Alvarez-Parrilla et al. (2007) studied the effects of the 4-hexylresorcinol (HR; 0.5 mM), *β*-CD (5 mM), and methyl jasmonate (MJ; 2 mM), and their combinations at the same levels as in the individual treatments on the oxidation of chlorogenic acid by PPO extracted form Red Delicious apples. They observed higher inhibition of the catalytic activity of the PPO when HR and *β*-CD were used together indicating a synergic effect. They suggested the synergic effect to be caused by different inhibition pathways: HR inhibits the oxidation by a competitive mechanism whereas *β*-CD inhibits oxidation by reducing the concentration of substrates with complexation. Any synergic effect was not observed between MJ and *β*-CD, which may have been due to the *β*-CD complexing MJ. However, Rosa et al. (2010) reported contrary results for the synergic inhibition effect of HR and *β*-CD. They studied the inhibition effects of HR (0.5 mM) and *β*-CD (10 mM) on PPO extracted from Prisco peaches with catechol, 4-methyl catechol, and chlorogenic acid as substrates. When HR and β-CD were used together, they observed a decreased inhibition activity as regards PPO oxidation of the chlorogenic acid compared to single inhibitor treatments.

The browning of fruits and juices can also occur non-enzymatically. Karangwa et al. (2012) studied the effects of HP-*β*-CD and *γ*-CD treatments on the non-homogenised and homogenised carrot-orange juices. They observed that the studied CDs had different effects on the non-enzymatic browning rates:

the HP-*β*-CD treatment significantly increased the non-enzymatic browning in both studied juice types and decreased the juice clarity, whereas the *γ*-CD treatment significantly decreased the browning in the non-homogenised juice. They concluded this phenomenon was caused by the increased solubility of carotenoids and other polyphenols by complexation with CDs.

2.2.3 Effects of the cyclodextrins on flavour properties

The unpleasant taste properties, such as bitterness and astringency of fruit and berry juices may limit their consumption. It is well known that certain phenolic acids and flavonoids cause bitter and astringent sensations in the oral cavity (Aron and Kennedy, 2008; Hufnagel and Hofmann, 2008; Sandell et al., 2009). Sugar has good masking properties, but excess consumption of sugar increases the risk of diseases, such as type II diabetes and obesity. The CDs can be used to reduce the bitterness of fruits, vegetables, and berries (**Table 1**, Deshaware et al., 2018; Konno et al., 1982; Mongkolkul et al., 2006; Shaw and Wilson, 1983). The effectiveness of the CDs is based on the formation of the inclusion complex between the CD and the bitter compound: when the bitter compound is bound to the CD cavity, the interaction between the compound and the bitter receptors is inhibited (Coupland and Hayes, 2014). Use of the CDs as binders of the bitter compounds, naringin and limonin, in citrus fruits have been widely studied. Konno et al. (1982) used 0.01–0.5 % of *β*-CD to bind naringin and limonin in the aqueous solution. In addition, they used 0.3 % and 0.5 % of *β*-CD in the amanatsu concentrate and 0.5 % in the heated and non-heated *Citrus iyo* juice. In the aqueous solutions, 0.5 % of *β*-CD reduced 50 % of the bitterness with both studied flavonoid compounds. *β*-CD significantly reduced the bitterness in both studied juices. Similar results have been reported with navel orange and grapefruit juices (Shaw and Wilson, 1983) and with Thai tangerine juice (*Citrus reticulate* Blanco; **Table 1**) (Mongkolkul et al., 2006). *β*-CD has also been used successfully to de-bitter bitter gourd juice (*Momordica charantia*; **Table 1**) (Deshaware et al., 2018). In the study, they were able to reduce the bitterness with low concentrations of CD, increasing the overall quality of the bitter gourd, but without effecting the colour or aroma properties.

Off- flavours can also be reduced with the CDs. Yang et al. (2020) studied the effect of *β*-CD and other polymers on the thermal treatment of water melon juice. They observed *β*-CD to be the most effective in reducing off-flavours after thermal treatment compared other studied polymers. In addition, they observed differences in the timing of the addition: addition of the *β*-CD after thermal treatment was more successful in reducing the contents of the off-flavour compounds compared to the treatment which *β*-CD was added before

thermal treatment. Lee et al. (2020) were able to reduce beany off-flavour compounds in the yuba film using isolated soy protein and the addition of *β*-CD. In addition, the effect of the removal of the *β*-CD after complexation was studied. An addition of 2 % and 4 % of β-CD significantly reduced the content of the compounds responsible for the beany flavour. In addition, these concentrations significantly reduced the beany flavour observed by a trained sensory panel. However, the total flavour strength decreased simultaneously with reduction of the beany flavour. Furthermore, they observed an increased level of *β*-CD decreased the puncture strength and deformation of the yuba film, but when the CD complexes were removed, there was no significant difference between the treatments. Similar results were reported by Suratman et al. (2004) in their study of the effects of *γ*-CD or *α*-CD on the beany flavour of two soymilks. They used 0.5 % (w/v) of *γ*-CD or *α*-CD and 0.25 % of both CDs. These CD contents significantly decreased the number of beany flavour compounds, but did not significantly reduce the beany flavour of the two soymilks as evaluated by trained sensory panel.

Off-flavours and undesirable tastes, such as bitterness, can be further eliminated when CDs are used with other inhibitors or maskers. This kind of synergic reduction of the undesirable flavours and tastes have been reported, for example, in goat cheese treated with *β*-CD and polymerised whey protein (Wang et al., 2018), (+)-catechin bitterness treated with *β*-CD and rebaudioside A (sweetener) (Gaudette et al., 2016), soy protein isolate treated with *β*-CD and phospholipase A_2 (Arora and Damodaran, 2011), and Alcalase salmon frame protein hydrolysate treated with *β*-CD and 2-butanol (Singh et al., 2020).

Volatile compounds effect the aroma of all food products. Different food processing methods, packaging materials, and storage conditions have an effect on flavour intensity, off-flavours, and volatile composition. CDs can be used to encapsulate volatile compounds, which improves the retention of these compounds (Decock et al., 2008; Reineccius et al., 2004; Tobitsuka et al., 2005) and, hence, improves the shelf-life of the food products and beverages. CDs are also used to increase the solubility of the volatile compounds, making them easier to use as flavouring agents (Kfoury et al., 2016). In addition, CDs, especially *β*-CD, can improve the retention of volatile compounds after an elevated temperature process. Reineccius et al. (2004) studied the effects of the encapsulated benzaldehyde, citral, *l*-menthol, and vanillin on the retention of these compounds and sensory properties in hard candies, fruit leather, and angel food cake. The use of *β*-CD improved the heat stability of the studied compounds. However, the result of sensory evaluation of the mint flavoured (*l*-menthol) hard candy, the citrus flavoured (citral) fruit leather, and the cherry flavoured (benzaldehyde) angel food cake showed that *β*-CD enhanced the flavour of citral and benzaldehyde compared to the liquid formulated flavour.

However, the liquid formulation of *l*-menthol in the hard candy resulted in a significantly more intensive minty taste than that encapsulated by the *β*-CD encapsulated. This study exhibited very well the differences in the inclusion complex stability between the compounds and the effects of the stability on the release of the guest compound.

2.2.4 Use of cyclodextrins in essential oils

Essential oils (EOs) have a long history as natural medications due to their natural antimicrobial properties. They are not strictly oils, but their poor water-soluble nature is similar to oils. The EOs are complex mixtures of hundreds of volatile aroma compounds, which provide their strong aromatic properties. Distillation, cold pressing, or extraction of the green plant parts, flowers, and fruits are typically used to produce EOs. (Calo et al., 2015) Many studies have proven that the use of the EOs can partly or entirely inhibit the growth of pathogenic bacteria in food (Cherrat et al., 2014; Costa et al., 2014; Lu et al., 2014; Roby et al., 2013). However, some studies have shown that the concentration of EOs necessary for antimicrobial activity is higher than the volatiles detection threshold, causing organoleptical changes in the food product (Firouzi et al., 2007).

The poor water-solubility of EOs is problematic for even dispersion in food matrices, and the compounds high volatility can make EO application difficult as a food preservative. The encapsulation of the volatile compounds have exhibited reduction of the evaporation, enhanced water-solubility, and even improved their antimicrobial properties. (Ayala-Zavala et al., 2008; Gaysinsky et al., 2007; Shah et al., 2013) Encapsulation can be performed, for example, with sodium caseinate (Pan et al., 2014), maltodextrin (Shah et al., 2013), or gum arabic (Cai et al., 2019), but *β*-CD can also be used (Anaya-Castro et al., 2017; Ayala-Zavala et al., 2008; Marques et al., 2019). Hill et al. (2013) encapsulated cinnamon bark extract, clove bud extract, *trans*-cinnamaldehyde, eugenol, and *trans*-cinnamaldehyde:eugenol mixture (2:1) with *β*-CD by the freeze-drying method, and determined the antimicrobial activity of the EOs and their *β*-CD complexes with the bacteria *Salmonella enterica* and *Listeria innocua*. They observed inhibition in the growth of both bacteria with the lower active compound concentration of all EO-*β*-CD complexes compared to free oils. In addition, the cinnamon bark and clove bud extract *β*-CD complexes were more powerful against the bacteria than the free volatile compounds or their mixture complexes. Liang et al. (2012) studied the antimicrobial activities of the individual volatile compounds of EOs (carvacrol, eugenol, linalool, and 2-pentanoylfuran) and their complexes with the *α*-CD, *β*-CD, and HP-*β*-CD. All the individual volatiles were effective against some of the studied

microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Saccharomyces cerevisiae*). Carvacrol was the most effective against all the microorganisms and had the lowest minimum inhibitory concertation (MIC; 0.5 µg/mL). Eugenol and linalool were effective against the Gram-positive bacteria *S. aureus* (MIC=2.5 µg/mL) and *B. subtilis* (MIC=1.25 µg/mL and 2.5 µg/mL, respectively). 2-pentanoylfuran was effective against *S. cerevisiae* (MIC=1.25 µg/mL). In addition, the CD complexation of the volatile compounds increased the antimicrobial activity towards all the studied microorganisms. Both studies concluded that the increased antimicrobial activity to be a consequence of the increased solubility of the volatile compounds. However, not all EO-*β*-CD complexes exhibit antimicrobial activity. Andrade et al. (2017) observed the antibacterial and modulatory-antibiotic activity of the *Hyptis martiusii* EO towards *S. aureus*, but no such effect was observed with the EO-*β*-CD complexes.

2.3 Enzymes in food processing

Enzymes are biochemical catalysts, which enhance biochemical or chemical reactions. The target molecules of the enzymatic reaction are called substrates, and they are converted into products via an enzymatic reaction. Enzymes are crucial for all biochemical processes to occur at the significant rate in nature. All catalysts lower the energy difference between the reactants and the transition state, thus lowering the activation barrier, which allows the reaction to be more rapid and enchancing the reaction rate. Enzymes are not consumed by the reaction and they do not change the equilibrium of the reactions. Enzymes are typically classified into six groups by the type of chemical reaction they catalyse: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. (Whitehurst and Oort, 2010a)

The utilisation of enzymes in any application requires knowledge of the basic enzyme kinetic theory as the enzymes can catalyse up to several million reactions per second in optimum conditions. With the enzyme kinetics, the binding of the substrate can be described, predicted, and calculated. In addition, the speed and efficacy of the substrate conversion to the product can be predicted.

Several parameters, such as the temperature and pH of the solution, the enzyme and substrate concentrations, the presence of the enzyme activators or inhibitors, and the effects on the enzymatic reaction rate (Bagger-Jørgensen and Meyer, 2004; Bora et al., 2017; Essa and Salama, 2002; Landbo et al., 2007; Mieszczakowska-Frąc et al., 2012a). Typically, an increased enzyme dosage, maceration time and temperature increases the juice yield and clarity (Essa and Salama, 2002; Landbo et al., 2007; Landbo and Meyer, 2004). Many studies

conclude that the enzyme concentration is the most important factor affecting the studied parameters (Landbo and Meyer, 2004; Lee et al., 2006; Liew Abdullah et al., 2007; Rai et al., 2004). The increment of the temperature increases the enzyme activity until the optimum temperature is reached. After that activity will decrease and eventually stop due to the enzyme denaturation (Handique et al., 2019). Lee et al. (2006) studied the effects of the temperature, enzyme concentration, incubation time, and the interactions of the parameters on banana juice. They observed that the increment of the temperature and its interactions with the enzyme concentration and longer incubation time had a positive effect on the clarity of juice. In their study, they concluded using a response surface methodology of 43.2 ºC to be the optimum temperature for the studied enzyme. Norjana and Noor Aziah (2011) observed that a higher pectinase concentration (0.1 %) with 3 h incubation resulted in the maximum juice yield of durian (*Durio zibethinus Murr*), but a pectinase concentration of 0.05 % with 3 h incubation resulted in the most acceptable sensory properties. However, changes in the parameters can cause unwanted changes in the juice. Landbo and Meyer (2004) observed that an increased maceration time reduced the anthocyanin contents of the black currant juice with some of the enzymes they studied. On the other hand, some of the enzymes increased the anthocyanin content when a longer maceration time was used. The differences are caused by the different side activities in the enzymes: high β-glucosidase side activity causes degradation of flavonoid glycosides, such as anthocyanins.

The production of enzymes is a very sophisticated procedure, which includes many individual unit processes. Even though plant cells secrete enzymes, such as pectinases and cellulases, the use of microorganisms to produce enzymes is the most efficient method. The microorganisms used can be natural enzyme producers, improved by classical techniques, or recombined with DNA techniques. Microorganisms must be safe to use, non-pathogenic and non-toxic, to produce enzymes for food and feed production. Many microorganisms can be used in enzyme production, but the bacteria belonging to *Bacillus* genera and filamentous fungi are the most utilised due to the following the microorganisms are easy to grow at fermenter at high biomass concentration, they are easily genetically manipulated to produce certain enzymes, they have good enzyme secretion characteristics, such as easy recovery of an enzyme from the broth, and they are recognised as safe hosts. (Sewalt et al., 2016; Whitehurst and Oort, 2010b)

2.3.1 Pectin and pectinolytic enzymes

Pectic substrates (pectins) are long and complex structural polysaccharides in the primary cell wall and middle lamella of plant cells. Pectins are linear and

composed from 1,4-linked α-D-galacturonic acids and esterified with methanol. The esterification degree varies between the plants, and it affects the jamming properties of pectin. (Coultate, 2002) As pectins are polysaccharides, they do not have a specific molecular weight, the size being dependent on the plant species. For example, the pectic substances in an orange have molecular weight between 40–50 kDa, whereas in an apple, the weight is between 200–360 kDa. (Jayani et al., 2005)

The pectinolytic enzymes (pectinases) form a heterogeneous group of the enzymes hydrolysing the α-1,4-glycosidic linkages in the pectic substrates. Pectinases can be divided into three groups: pectin methyl esterases (EC 3.1.1.11), polygalacturonases (EC 3.2.1.15), and pectic lyases (EC 4.2.2.10). Pectin methyl esterases removes the methanol residues from the methyl galacturonate residues. Polygalacturonases and pectic lyases hydrolyse the glycosidic links between the galacturonate residues: the polygalacturonases degrade unmethylated and the pectic lyases methylated galacturonates. There are two types of polygalacturonases: *exo*-polygalacturonases, which hydrolyse the small chain fragments from the non-reducing end of the pectin chain, and endo-polygalacturonases, which hydrolyse the pectin substrate within the chain (Coultate, 2002).

Pectinases are the most utilised enzymes in the beverage industry due to their wide beneficial effects on the final product. Because the plant cell walls are highly complex structures, the commercial enzyme products are composed of many enzymes, such as pectinases, cellulases, and hemicellulases, together with different target polymers. Some pectines have very complex structures, and need more than one kind of pectinase to break it down. The effects of the pectinases on the chemical composition, rheology, and sensory properties of berry and fruit juices have been widely studied (Laaksonen et al., 2012; Lee et al., 2006; Liew Abdullah et al., 2007; Puri and Banerjee, 2000; Sandri et al., 2013; Versari et al., 1997). The clearest and, for the producers, the most desired effect is an increased juice yield. The corresponding values of yield before and after pectinase treatments are for banana 47.3 % and 71.4 %, respectively (Bora et al., 2017), bilberry 66.8 % and 78.7–79.8 % (Buchert et al., 2005), black currant 70 % and 77 % (Laaksonen et al., 2013), prickly pear 66.8 % and 87.8 % (Essa and Salama, 2002), and pineapple 62.8 % and 80.1 % (Sreenath et al., 1994).

The degradation of pectin not only releases the juice, but it also releases the chemical compounds from the cell matrix. However, commercial pectinase enzymes may have many side activities, such as different glycosidase activities, which may also have an effect on the final product. Versari et al. (1997) reported both a decrease and an increase of flavonol glycosides after pectinase treatments. They noted that the difference was dependent on the enzyme

product used, indicating different side activities occurring in the enzyme products.

Pectinase treatments have been reported to effect the sensory properties of black currants by increasing the sourness, astringency, and fermented odour and flavour due to the increased contents of proanthocyanidins, flavonols, and hydroxycinnamic acids (Laaksonen et al., 2012). However, the sensory properties of black currant nectar treated with pectinase have been reported to be unaffected by the enzymatic treatment (Iversen et al., 1998). Pectinase treatment has increased the overall acceptance of durian juice by increasing the sweetness and aroma (Norjana and Noor Aziah, 2011).

2.3.2 Cellulose and celluolytic enzymes

Cellulose is one of the most abundant biomaterials in the world and all plants synthesise it. It is only formed from glucose units via *β*-1,4-linkages making it a homopolymer. A cellulose chain is linear, and it can contain hundreds to thousands of glucose units. The chains are intertwined together forming the crystal structure, microfibrils, which can further form macrofibrils. This crystal structure makes cellulose tough, fibrous, and insoluble to water. It has a crucial role keeping the plant cell wall structure stable. (Brigham, 2018; Jayasekara and Ratnayake, 2019)

Celluolytic enzymes (cellulases) forms an enzyme group, which catalyses the degradation of the cellulose into glucose units. Cellulases use acid-base catalysis to break down the *β*-1,4-glycosidic bonds, using two different mechanisms: the retention and inversion of the anomeric configuration of cellulose. The cellulase group includes three types of enzymes: *endo-β*-1,4-glucanases (EC. 3.2.1.4), *exo-β*-1,4-cellobiohydrolases (EC. 3.2.1.91), and *β*-glucosidases (EC. 3.2.1.21). Endoglucanase degrades the amorphous areas of cellulose, creating new chain ends. Exoglucanase unbinds the glucose or cellobiose units from the reducing or non-reducing end of the cellulose chain and it is usually highly active against crystalline cellulose. *β*-glucosidases can form glucose units from the non-reducing end of the cellobioses. Most animals cannot synthesis cellulases, and commercial cellulases are produced by fungi and bacteria. In the beverage production, cellulases are used to hydrolyse cell wall polysaccharides and substituted cellulose. This will increase the juice yield and help to clarify the juice. Cellulases are also utilised, for example, to decrease the viscosity of nectars, to concentrate purees, to extract oil from oil seeds, improve the water absorption of the cereals (Jayasekara and Ratnayake, 2019; Sharma et al., 2017).

2.3.3 *β*-glucosidases

β-glucosidases are a wide group of enzymes, which hydrolyse glycoside bonds, releasing non-reducing terminal glucosyl residues from oligosaccharides and glycosides. *β*-glucosidases are ubiquitous and foun in all living cell. They have avariety of functions, such as glycolipid and exogenous glucoside breakdown in animal cells, biomass conversion in microorganisms, and oligosaccharide catabolism. In addition, they release scents from their glycoside storage forms in plant cells. Substrate specificity groups *β*-glucosidases into three groups: aryl-*β*-glucosidase, cellobiases, and broad speficity *β*-glucosidases. The aryl-*β*-glucosidases have an extreme specificity towards aryl-glycosides, such as phenyl and xylyl. The cellobiases only hydrolyse cello-oligosaccharides, such as cellobiose. The third group includes the *β*-glucosidases with significant activity towards both substrate types. This group is the most presented in the celluolytic microbes. *β*-glucosidases are also grouped in glycoside hydrolase (GH) families and further subfamilies and clans by their amino acid sequence similarities (www.cazy.org). For example, the GH1 includes enzymes, among others, with *β*-glucosidase, *β*-galactosidase, and phlorizin hydrolase activities. (Ketudat Cairns and Esen, 2010; Yeoman et al., 2010)

2.3.4 Effects of enzymatic treatments on the chemical composition and sensory properties of fruit and berry juices

Enzymes are widely used in the food producing, e.g. in the manufacture of beverages, cheese, oils, and bakery products. However, due to the high applications of enzymes, this thesis will concentrate only on the effects of pectinasaes, cellulases, and *β*-glucosidases on juices and fermented beverages. The effects of the enzymatic treatments on the chemical composition and sensory properties of fruit and berry juices are shown in Table 2. Fruits and berries contain more than 80 % moisture, which classifies them as highly perishable commodities: 25 % of fruits and vegetables are lost during post-harvest, processing, distribution, and consumption. Consumption causes the largest amount of waste fruits and vegetables compared to all other parts of the food supply chain (agriculture excluded) (De Laurentiis et al., 2018; Gustavsson et al., 2011). Juicing of fruits and berries is a common way to preserve them and increase the shelf life (Sharma et al., 2017). Processing of the juices includes many unit operations, such as sorting, washing, and chopping of the raw material, juice extraction (enzymatic, heat or cold extraction), pressing, clarification, and filtration. In addition, the juice can be concentrated or pasteurised. Enzymes can be use in many unit operations during juice production. The commercial enzyme products for juice processing

typically contain pectinolytic and cellulolytic enzymes that degrade the polysaccharides forming the cell wall network and releasing the juice and glycosidic bound compounds. The high pectin content is a primary hindrance in the filtration due to the formation of a highly viscous gel on the surface of the membrane filters. Decreased viscosity improves pumping, filtering and packaging properties thus making it an important consequence from an industrial and processing point of view. (Sinha et al., 2012) Moreover, enzymes are typically utilised in beverage production to yield more stable and clarified products and to achieve more acceptable products. Pectinase treatment can significantly reduce the turbidity and viscosity of the final juice, and reduce the precipitate content. Lachowicz et al. (2019) studied the effects of nine pectinase enzymes on the comprehensive quality of chokeberry juice before and after five-months storage at 5 °C. They observed significant reduction in both turbidity and viscosity. In addition, there were significant difference in the turbidity between the juices treated with different pectinases.

Table 2. The effects of the enzymatic treatments on the chemical composition and sensory properties of fruit and berry juices.

Publication	Raw material	Enzymes	Incubation method	Focus	Effects on compounds important for sensory properties
Iversen et al. (1998)	Black currant nectar	A pectinase	Dosage: 0.0057 % enzyme with 8800 PG/mL activity at 3.5 pH. Incubation for 2 h at 50 °C, pasteurisation for 27 s at 88 °C	Volatile compounds	Major degradation of esters during enzyme treatment, pasteruisation did not have significant effect on volatiles
Buchert et al. (2005)	Bilberry black currants	A cellulase Four pectinases	2h at 45 °C	Total and monomeric anthocyanins	Increase in the total content of anthocyanins, decrease in galactosides of anthocyanidins.
Koponen et al. (2008)	Black currants and bilberries and press residues	A cellulase Three pectinases	Dosages: 0, 1, 10, and 100 nkat/g or 0, 1, 10, and 20 nkat/g. Incubation for 2 h at 45 °C.	Effects of enzyme dosage on the total flavonols and monomeric flavonols.	Increase in total flavonol content in the juice and decrease in the press residue. Extraction of flavonols from black currant were dependent on the higher dosage of enzyme and bilberry more on the used enzyme.
Koponen et al. (2008)	Black currants bilberries press residues	A cellulase Three pectinases	Dosages: 0, 1, 10, and 100 nkat/g or 0, 1, 10, and 20 nkat/g. Incubation for 2 h at 45 °C.	Effects of enzyme dosage on the total anthocyanins and monomeric anthocyanins.	Affected more on the total anthocyanin content of black currant juice. Used enzyme and dosage level had effect on the total anthocyanin yields and profile in both juices. Extraction of anthocyanin from black currant was more depended on enzyme.
Puupponen-Pimiä et al. (2008)	Bilberry	Nine pectinases	Dosage: EPG activity 100 nkat/g for centrifuged juice and 10 and 50 nkat/g for pressed juice. Incubation for 2 h at 45 °C.		In both cases, all treatments increased total phenolic and anthocyanin contents (except one enzyme). Higher dosage increased the phenolic content in the press cake.

Publication	Raw material	Enzymes	Incubation method	Focus	Effects on compounds important for sensory properties
Laaksonen et al. (2013)	Black currant, five varieties	A pectinase	Dosage: 150 mg of enzyme per one kg of berry mash. Incubation for 4 h at 45-47 °C.	Sugars and organic acids, monomeric anthocyanins, flavonols, and phenolic acids	Enzymatic treatment increased organic acid contents in all juices and decreased sugar/acid ratio. Sugar contents after treatment were depended on berry variety. Enzymatic treatment increased almost all contents of analysed phenolic compounds. Phenolic acid contents were least effected. Treatments increased the intensities of astringency and bitterness.
Dinkova et al. (2014)	Bilberry	Three pectinases	Dosage: 0.1 % (v/v). Incubation for 2 h at 50 °C and pressing, or incubation for 2 h at 50 °C after partial de-juicing (40 %). Refrigerated storage at 10 °C for 9 d.	Total phenolics and anthocyanins, monomeric anthocyanins, and antioxidant capacity	Increase of total phenolic and anthocyanin contents and antioxidant capacity. Decrease of certain galactosides of anthocyanidins.
Heffels et al. (2017)	Bilberry	Two cellulases Four pectianses	Dosage: 0.5 nkat/g and 10 nkat/g. Incubation for 120 min at 50 °C.	Total anthocyanins and anthocyanin profile	Incrase of total anthocyanin contents. Anthocyanin profile depended on the used enzyme and used dosage: in higher level, profile was more affected by side activities of enzymes.
Mäkilä et al. (2017)	Black currant	A pectinase	Press residue of NEB: Dosage: 205 mg of enzyme per one kg. Incubation for 4 h at 45–47 °C. EB juice: Dosage: 150 mg/kg of berry mash. Incubation 4 h at 45–47 °C. After pressing: Degassing and pasteurisation at 95–97 °C for 30 s. Storage for 12 mon at RT in light/dark and + 4 °C in dark.	Sugars and organic acids, monomeric anthocyanins, flavonols, and phenolic acids	Juice from press residue of NEB had the highest contents of all analysed compounds (not sugars and citric acid methyl esters). Enzymatic treatment effected on the chemical composition of the juices after storage. Cold storage decreased the degradation of phenolic compounds.

Publication	Raw material	Enzymes	Incubation method	Focus	Effects on compounds important for sensory properties
Tian et al. (2017)	Flat pear	A β-glucosidase	Pasteurised juice was treated with β-glucosidase (1.36 U/mL) at 45, 55, or 65 °C for 60 min.	Sensory properties, volatile profile	Enzymatic treatments decreased cooked and unnatural flavour and increased the floral, fruity, and peach-like flavours compared to pasteurisation. Volatile profile was affected by the enzymatic treatments.
Marsol-Vall et al. (2019)	Black currant	A pectinase	Incubation for 2 h at 40–50 °C. Storing in the dark at +4 °C in refrigerator, in the dark at room temperature, under light at room temperature	Volatile compounds	Pasteurisation had mainly increasing effect on the volatiles on NEB and decreasing on EB. Enzymatic treatment decreased the volatile content.

EB enzymatic treated beverage; EPG endopolygalacturonase; FRSA free radical scavenging activity; NEB non-enzymatic treated beverage; RT room temperature; TPC total phenolic content

Enzymatic treatments can also chemically stabilise the final juice product. Dinkova et al. (2014) observed an increased half-life of the bilberry anthocyanins after enzymatic treatments during a nine-day chilled storage period compared to the non-enzymatically treated bilberry juice. Mäkilä et al. (2017) reported similar results for black currant anthocyanins after a 12 months chilled storage (**Table 2**). They also studied effects on the contents of hydroxybenzoic acids, flavonol glycosides, flavonols, hydroxycinnamic acid derivatives, and hydroxycinnamic acids. They did not observe notable different in the contents of flavonol glycosides, flavonols, and hydroxycinnamic acid derivatives after 12 months of chilled storage. However, they observed an increase in the hydroxycinnamic acid and hydroxybenzoic acid contents in the non-treated juice and non-conventional enzymatic treated juice, but not in the conventional enzymatic treated juice after storing. The storage at room temperature resulted in more drastic increase in hydroxybenzoic acid and hydroxycinnamic acid and sharper decrease in anthocyanins in all samples.

During pectinase and cellulase treatments the water-soluble and insoluble cell wall components are degraded and the polyphenols bounded to them are released into the aqueous solution (Bagger-Jørgensen and Meyer, 2004; Buchert et al., 2005). The increased content of the polyphenols not only effects the antimicrobial and antioxidant activities in the juice (Dinkova et al., 2014; Puupponen-Pimiä et al., 2008; **Table 2**), and the colour properties, but also may cause unwanted quality factors in the juice, especially unwanted sensory properties. As previously reported with black currants (Laaksonen et al., 2013, 2012), the enzymatic juices may have more astringent, bitter, and sour sensory profiles due to the higher contents of flavonoids (**Table 2**). However, Viljanen et al. (2014) reported lingonberry juice to be sweeter after enzymatic treatment compared to the untreated, lactic acid and yeast fermented samples.

Raw material may affect the colour properties of enzymatically pressed juice. For example, the colour of the chokeberry (Lachowicz et al., 2018) and blueberry (Siddiq et al., 2018) juices can be improved with pectinases, whereas the enzymatic treatment of lingonberry (Viljanen et al., 2014) decreases the colour intensity. The differences between the juices produced from the anthocyanin containing berries may have be due to the differences in the monomeric anthocyanin composition and the different side-activities in the commercial enzyme products. Such berries as lingonberries, which contains only few major anthocyanins, are more sensitive to the changes in the total and monomeric anthocyanin contents caused by the enzymatic treatment, that is the enzyme product contains high glucosidase or galactosidase side-activities (**Table 2**). (Buchert et al., 2005; Viljanen et al., 2014)

Some enzymes are directly used to modify taste properties. Naringinase (EC 3.2.1.40) is one kind of glucoside hydrolase (glucosidase) the main activities of

which are *α*-L-rhamnosidase and *β*-D-glucosidase. It can hydrolyse many glycosides, such as naringin, quercetin, and rutin. Naringin (4',5,7-trihydroxyflavanone-7-rhamnoglucoside) is a flavanone glycoside occurring in citrus fruits. It has peculiar bitter taste with a low detection threshold. Naringinase hydrolyses naringin to rhamnose and prunin, which is further hydrolysed to glucose and naringenin, which is an almost tasteless flavanone, by *β*-glucosidase. (Ribeiro, 2011) In the beverage industry, naringinase is used to de-bitter citrus fruit juices. Prakash et al. (2002) studied different naringinase treatment conditions and their effects on the naringin content and bitterness in Indian grapefruit (*Citrus paradise*). They observed that an increase of the naringinase concentration, temperature, and incubation time reduced most naringin content. Enzyme treatment with a naringinase concentration of 1.0 g/L, incubated at 40 ºC for 4 h, resulted in a maximal debittering of the order of 75 %. The sensory panel evaluated this juice organoleptically acceptable. Ni et al. (2015) studied the effects of naringinase on the aroma properties of pumelo (*Citrus grandis*) juice. They observed a more complex volatile compound profile after naringinase treatment than in the non-treated sample. Sensory evaluation exhibited less citrus flavour, but more sweet, floral, green, mushroom, and cooked flavours in the naringinase treated sample.

Enzymatic treatments also release the glycoside bound volatiles. This may lead to changes in the original aroma of the fresh berries, resulting in the juices with less berry-like and more concentrate juice-like odour (Kim and Park, 2017; Laaksonen et al., 2013; Marsol-Vall et al., 2019a; Mieszczakowska-Frąc et al., 2012b). However, Tian et al. (2017) observed *β*-glucosidase treatment with moderate temperature of flat peach resulted in a more peach-like, floral, and fruity juice compared to only pasteurised juice (**Table 2**). Shoseyov et al. (1990) used immobilised *endo-β*-glucosidase to treat passion fruit juice and Muscat Roy wine. In both cases, they observed an increment in the volatile compounds, such as linalool, *α*-terpineol, and benzaldehyde. The flavour of passion fruit juice was increased significantly after *β*-glucosidase treatment. In addition, 11 out of 11 panellists was able to identify the treated passion fruit juice from the untreated and 7 out of 11 the treated wine sample in the duo trio experiment.

2.4 Alcoholic fermentation

Wine is significantly different from the original raw material when chemical composition and sensory properties are compared. Alcoholic fermentation includes a series of the biochemical reactions, aiming for release of energy from the sugars. At the same time, yeast metabolises amino acids and other nitrogen sources, and to form co-enzyme A and new amino acids for its own use. These biochemical reactions lead to the formation of ethanol, glycerol,

organic acids, higher alcohols and their acetates, hydrogen sulphides, and fatty acids and their esters, respectively.

The alcoholic fermentation releases energy from the sugars forming ethanol as its main by-product. Most organisms can ferment sugars, but fermentation is usually exploited only when oxygen is lacking due to its ineffective energy release and toxic by-products (ethanol, lactic acid). The alcoholic fermentation releases only 6 to 8 % of the chemical-bond energy. In other words, alcohol fermentation produces only two adenosine triphosphate (ATP) molecules from each glucose molecule, whereas respiration produce 36 to 38 ATPs from each glucose molecule. (Waterhouse et al., 2016) Yeast bonds energy to ATP using only glycolysis in an anaerobic environment (**Figure 4**), which is same in both aerobic respiration and anaerobic alcoholic fermentation. Yeasts are able to use respiration in the aerobic environment, but in high glucose concentrations, yeasts primarily exploit fermentation because glucose represses the gene expressions coding the respiration enzymes (Horák, 2013). Glycolysis starts with the glucose conversion to fructose 1,6-biphosphate, which is further cleaved into two triose phosphate isomers, glyceraldehyde 3-phospahe (G3P) and dihydroxyacetone phosphate, by aldolase. The latter one is isomerised to G3P, resulting two molecules of G3P from one glucose molecule. The next step recovers the energy from G3P by the formation of 3-phosphoglycerate and ATP. The last step of glycolysis transforms 3-phosphoglycerate into pyruvate and ATP. Hence, four ATPs are recovered from one glucose molecule, but two of them are immediately used to start new glycolysis, so the net gain is two ATPs per one glucose molecule. Alcoholic fermentation adds a two enzyme catalysed reaction to the glycolysis: first, the pyruvate carboxylase forms acetaldehyde and CO_2 from the pyruvic acid, and second, alcohol dehydronase reduces the acetaldehyde to ethanol with NADH. (Ribéreau-Gayon et al., 2006)

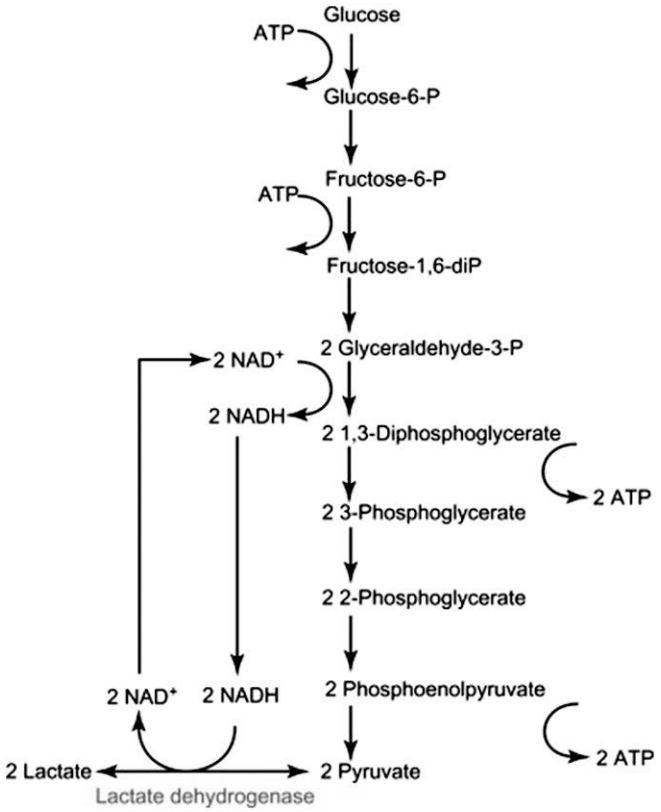

Figure 4. Biochemical pathway of glycolysis (Feher, 2017).

At the beginning of the alcohol fermentation or in at high sulphite environment, the fermentation of the glucose also produces glycerol by the glyceropyruvic fermentation. A high sulphite content causes acetaldehyde to take its bisulphite form, which cannot be reduced to ethanol, making dihydroxyacetone-1-phosphate the terminal electron acceptor. Dihydroxyacetone phosphate is reduced to glycerol-3-phosphate, which is further dephosphorylated into glycerol. When alcoholic fermentation starts, the fermentation environment consists of more oxygen. In addition, pyruvate decarboxylase and alcohol dehydrogenase are still weakly expressed. Acetaldehyde does not accumulate, so re-oxidation of NADH is done by the dihydroxyacetone. This leads to the formation of glycerol, pyruvate, and secondary fermentation products, such as succinic acid, α-ketoglutaric acid, diacetyl, and butanediols, from pyruvate. (Ribéreau-Gayon et al., 2006)

The raw material and the fermentation conditions have significant impacts on the yeast adaption, growth rate and the quality of the final wine. The sugar and nitrogen contents and temperature directly effect the final ethanol content, pH, total acidity, and nutrients, such as the composition of nitrogen and lipids. (Escribano-Viana et al., 2020; Su et al., 2020) Escribano-Viana et al. (2020)

studied the oenological changes after fermentation of three red grape varieties with *S. cerevisiae*, *M. pulcherrima*, and a simultaneous fermentation with *Lachancea thermotolerans-T. delbrueckii*. They observed different yeast adaption and alcoholic fermentation rates for all the grape varieties. In addition, the oenological and colour parameters were more dependent on the grape variety. In contrast, the aroma profiles showed more dependence on the yeast species. Lu et al. (2018) investigated the effects of the sugar concentration (°Brix 17, 23, and 30) of mango juice on the final mango wine composition. The fermentations were performed with a *S. cerevisiae* yeast. Low sugar content had a positive effected on the growth rate, cell population, and volatile compound profile, but also caused an increment in the fatty acid concentrations, which may have a negative effect on the flavour of the wine. The medium and high sugar contents significantly decreased the acetate and ethyl ester concentrations.

Nitrogen is an essential nutrient for yeast during alcoholic fermentation. Compared to the other nutrients, the concentration of yeast assimilable nitrogen (YAN) is typically low in the must, thus, it often limits both the yeast growth and fermentation rate. This can cause a sluggish or stuck fermentation and formation of off-flavours. Yeast uses ammonium and amino acids as a nitrogen source to produce proteins, enzymes, and structural components (mannoproteins). (Waterhouse et al., 2016) The amino acid composition has an impact on the formation of the volatile compounds during the fermentation. It is well known that certain higher alcohols are formed from certain amino acids (Dickinson et al., 1998; Hernández-Orte et al., 2002; Seguinot et al., 2020; Su et al., 2020). For example, 2-methyl-1-propanol, 2-methylpropanoic acid, 2-methylpropyl acetate, and ethyl butyrate are directly derived from valine, and methionol is derived from methionine. However, the ^{13}C-labelation of the glucose has shown that more than 75 % of the higher alcohols are produced by the anabolic pathway from the glucose (Nisbet et al., 2014). In addition, volatile compounds are not produced at the same rate: from 1 µM of methionine 0.64 µM of methionol is produced, but only 0.11 µM of leucine related compounds (3-methyl-1-butanol, 3-methylbutyl acetate, 3-methylbutanoic acid, and ethyl 3-methylbutanoate) were produced from 1 µM leucine. (Seguinot et al., 2020)

2.4.1 Wine yeasts

Wine yeasts have some essential characteristics in the production of quality wine. Wine yeast must be able to grow in a high sugar content and low pH and tolerate SO_2 or chitosan, copper, and oxidative stress. In addition, yeast must have certain metabolic traits, such as certain enzymatic properties, resistance

against 5,5′,5″-trifluoro-D-leucine or cerulenin, which effect the organoleptic properties of final wine. (Waterhouse et al., 2016)

2.4.1.1 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is the most utilised yeast with a history of over 5000 years (Cavalieri et al., 2003). *S. cerevisiae* is a very familiar yeast, which has many established positive characteristics, facilitating its high status in alcoholic fermentation. *S. cerevisiae* has a good fermentation performance in high sugar and low pH environments. The optimum pH ranges between 4.5 and 6.5 and the temperature between 20 and 30 °C. Some *S. cerevisiae* strains tolerate up to 18 % of ethanol content. It has killer yeast activities, ensuring competitive advantage against other yeast and bacteria and the sole right to resources (Replansky et al., 2008). *S. cerevisiae* requires high water activity (a_w minimum ~0.65) for a good fermentation performance. In the high sugar fermentations, a_w decreases, causing osmotic stress on the yeast cell. The stressful situation triggers production of osmolytes, such as glycerol and trehalose. This may lead to the over-production of glycerol and so reduce the ethanol content. Even though *S. cerevisiae* preferes anaerobic conditions and is referedred to a facultative anaerobe, strict anaerobic conditions stops its growth due to the fact that *S. cerevisiae* needs oxygen to produce membrane fatty acids and sterols. (Walker and Stewart, 2016)

2.4.1.2 *Saccharomyces bayanus*

S. bayanus is remotely related to *S. cerevisiae* and other *Saccharomyces* species. *S. bayanus* is the second most important wine yeast after *S. cerevisiae* due to its good fermentation performance, but it still has different metabolic characteristics and environmental requirements compared to *S. cerevisiae*. *S. bayanus* is a cryophilic with an optimum temperature range between 8 and 10 °C, which may explain its characteristic glycerol accumulation. (Naumov et al., 2011) Generally, *S. bayanus* produces more glycerol, succinic acid, and higher alcohols and denser colour than *S. cerevisiae*. Furthermore, it produces less ethanol, ethyl and acetate esters, acetic acid, and volatile fatty acids and consumes less malic acid compared to *S. cerevisiae* (Blazquez Rojas et al., 2012; Eglinton et al., 2000; Tosi et al., 2009). *S. bayanus* exhibits active transportation *via* cell walls for fructose contrary to *S. cerevisiae*. Table 3 presents main effects of *S. bayanus* fermentations compared to *S. cerevisiae*.

Table 3. The main characteristics (effect) of *S. bayanus* fermentation compared to *S. cerevisiae* fermentation.

Reference	Raw material	Strain	Main effects*
Satora et al. (2018)	Apple	DSMZ 3774	Free amino nitrogen ↑
			Titratable acidity ↑
			Ethanol ↓
			Malic acid ↑
			Higher alcohols ↑
			Volatile esters ↑
			Carbonyl compounds ↑
			Aroma ↑
Duarte et al. (2010)	Raspberry	CBS 1505	Ethanol ↓
			Methanol ↑
			Succinic acid ↓
			Hydroxycinnamic acids ↓
			Acetates ↓
			Volatile phenols↓
			Fatty acids ↓
			Volatile sulphur compounds ↑
Liu et al. (2017)	Mulberry	RW	Ethanol ↑
			Monomeric anthocyanins ↓
			Total phenols ↑
			Co-pigmented anthocyanin ↓
			Colour intensity ↓
		SY	Total phenols ↑
			Total flavonoids ↑
			Co-pigmented anthocyanin ↓
			Colour intensity ↓

↑ increase in content; ↓ decrease in content

2.4.1.3 *Torulaspora delbrueckii*

Torulaspora delbrueckii is the most studied and utilised non-*Saccharomyces* wine yeast. It most resembles *S. cerevisiae* with a good fermentation performance, sugar utilisation, killer yeast activities, and metabolism regulation patterns. (Comitini et al., 2011; Ramírez and Velázquez, 2018; Velazquez et al., 2015) Table 4 presents studies of the pure *T. delbrueckii* fermentation, and sequential or simultaneous fermentation with *S. cerevisiae* compared to pure *S. cerevisiae* fermentation. In the traditional, high sugar grape must fermentation, *T. delbrueckii* is usually used in the simultaneous or sequential fermentation with *S. cerevisiae* due to its low ethanol tolerance (9–10 %, v/v) (Bely et al., 2008). Furthermore, *T. delbrueckii* has slower growth and fermentation vigour in the anaerobic fermentation compared to *S. cerevisiae*, leading to the *S. cerevisiae* being overcome in the simultaneous/sequential fermentation.

The simultaneous/sequential fermentation with *T. delbrueckii* improves the comprehensive quality of the final wine, including a more complex aroma profile, decreased acetic acid and volatile acid concentrations, and an increased glycerol concertation. Increased production of ethyl propanoate, ethyl isobutyl, and ethyl dihydrocinnamate have been linked to the maximal population of *T. delbrueckii* in the simultaneous fermentation with *S. cerevisiae*. In addition, these two yeasts together have exhibited positive interactions towards the production of isobutyl acetate and isoamyl acetate. The increse of the aforementioned esters generates more *fruity* and *complex* taste properties. (Renault et al., 2015) Simultaneous and sequential fermentations may result in very different wines. Loira et al. (2015) studied simultaneous and sequential fermentations with three *S. pombe* or three *T. delbrueckii* strains and *S. cerevisiae*. They observed significant difference in the monomeric, acetylated, and coumarylated anthocyanins, vinylphenolic pyranoanthocyanins and vitisins between simultaneous and sequential fermentations. Generally, simultaneous fermentations have higher concentrations. In addition, the volatile compound profiles were markedly different between the fermentation types, the simultaneous fermentation exhibiting higher volatile concentrations.

Table 4. The characteristics (effect) of *T. delbrueckii* fermentation compared to *S. cerevisiae* fermentation.

Reference	Yeast	Strain	Raw material	Main effects*
Hu et al. (2020)	*T. delbrueckii* SeqF.		Citrus fruit (Ponka)	Ethanol ↓ Titratable acidity ↓ Fatty acids ↑ pH ↑ Higher alcohols ↑ Acetates ↑ Total esters ↑ Terpenes ↑ Sensory properties ↑
Liu et al. (2019)	*T. delbrueckii*	291	Bilberry	Ethanol ↓ Esters ↓ Aldehydes ↓ Benzenes ↓
		70526		Ethanol ↓ Higher alcohols ↑ Monoterpenes ↑ Aldehydes ↓ Benzenes ↓
	T. delbrueckii SeqF	291		Ethanol ↓ Aldehydes ↓ Acetals ↓ Benzenes ↓
		70526		Higher alcohols ↑ Aldehydes ↓ Acetals ↓ Benzenes ↓
	T. delbrueckii SimF	291		Aldehydes ↓ Acetals ↓
		70526		Higher alcohols ↑ Aldehydes ↓ Acetals ↓
Liu et al. (2020)	*T. delbrueckii* SeqF		Kiwi	Ethyl esters ↓ Other esters ↓ Fatty acids ↓ Ketones ↑ Volatile phenols ↓
	T. delbrueckii SimF			Ethyl esters ↑ Other esters ↓ Fatty acids ↑
Sun et al. (2014)	*T. delbrueckii*	Zymaflore	Cherry	pH ↑ Titratable acidity ↑ Esters ↓ Higher alcohols ↓ Fatty acids ↓
	T. delbrueckii SeqF			Esters ↑ Higher alcohols ↑ Fatty acids ↓ Terpenoids ↑

*compared to *S. cerevisiae* fermentation; ↑ increase in content; ↓ decrease in content; ↔ same level; HA hydroxycinnamic acids; SimF. simultaneous fermentation with *S. cerevisiae*; TA titratable acidity; SeqF sequential fermentation with *S. cerevisiae*

T. delbrueckii is used to produce wines with reduced ethanol content. Co-fermentation with *T. delbrueckii* may lead to as high as 1 % reduction in the ethanol content compared to the pure *S. cerevisiae* fermentation (Azzolini et al., 2015; Canonico et al., 2019; Contreras et al., 2014), however, the use of some *T. delbrueckii* strains do not reduce the ethanol content (Loira et al., 2015; Renault et al., 2015). Rêgo et al. (2020) observed that pure fermentation of cashew apple juice with *T. delbrueckii* alone had a significantly lower ethanol level than the *S. cerevisiae* fermented juice, but the simultaneous fermentation (1:1) did not result in a significantly reduced ethanol level. Fermentation with *T. delbrueckii* alone has shown low undesirable volatile acidity, higher alcohols, glycerol, and ethanol production compared to *S. cerevisiae* fermentation (Bely et al., 2008; Einfalt, 2020). However, the study by Einfalt (2020) observed that a lower content of higher alcohols negatively effected on the body perception of barley-sorghum beer. In addition, *T. delbrueckii* has shown low ethyl and acetate ester production in grape and fruit wines (**Table 4**), which may decrease the fruit and floral attributes (Liu et al., 2020; Liu et al., 2019; Renault et al., 2015; Sun et al., 2014). Hu et al. (2018) studied the effects of nine different non-*Saccharomyces* yeasts on the volatile composition and sensory properties of orange wine. *T. delbrueckii* fermented wine had the lowest total volatile compound content, though some volatiles were higher, and even some new ones appeared, compared to *S. cerevisiae* fermented wine. Nonetheless, they observed *T. delbrueckii* to significantly improve the mouthfeel, taste lasting, and the total sensory score of the orange wine compared to *S. cerevisiae* fermented wine. Liu et al. (2020) observed that sequential fermentation with *T. delbrueckii* and *S. cerevisiae* resulted in a significant decrease in the *solvent*, *sour*, and *herbaceous* flavours, which are considered as off-flavours, and an increase in the *fruity* aroma on the global aroma of the malolactic fermented black raspberry wine compared to the pure *S. cerevisiae* fermented wine.

2.4.1.4 *Metschnikowia pulcherrima* and *M. fructicola*

Metschnikowia yeasts are ubiquitous. They are found in the spontaneous wine fermentations and on the surfaces of many fruits. Their fermentation performance and ethanol tolerance are low (3–9 %) (Barbosa et al., 2018; Comitini et al., 2011; Morales et al., 2015). Therefore, *Metschnikowia* yeasts are typically used together with *S. cerevisiae* in sequential fermentation, which is started with *Metschnikowia* yeast. They take part in the beginning of the fermentation and their growth decrease when the ethanol level increases. In addition, *M. pulcherrima* and *M. fructicola* have shown killer yeast activities (Kurtzman and Droby, 2001). Some *M. fructicola* strains are principally used to

preserve the wine must due to the vigour of their killer yeast activities (Lallemand Oenology, 2017).

Many studies have reported fermentation with *Metschnikowia* yeasts resulting in wine with a more complex aromatic profile (Boscaino et al., 2019; Duarte et al., 2019; Lee and Park, 2020; Suárez-Lepe and Morata, 2012). *M. pulcherrima* has a characteristic *β*-glucosidase (Fernández et al., 2000) and *α*-L-rhamnosidase activity (Comitini et al., 2011). Non-volatile glycosides of aroma precursors are common in fruits due to their better water solubility compared to the aglycon forms. Glycosidases cleave sugar moieties from aroma precursor glycosides contributing to the complexity of the wine aroma. (Suárez-Lepe and Morata, 2012) In addition, some *M. pulcherrima* and *M. fructicola* strains are reported to have polygalacturonase activity (Belda et al., 2016; Fernández et al., 2000), which may increase the extraction of flavonoids, such as anthocyanins, and so increase the quality of the final wine. On the other hand, the excess extraction of flavonols and flavan-3-ols may increase the intensity of bitterness and astringency (Hufnagel and Hofmann, 2008). *M. fructicola* has been reported to effect the volatile composition of the wine, by increasing the acetate and ester formation, when used together with *S. cerevisiae* (Boscaino et al., 2019).

Due to low fermentation performance of *Metschnikowia* yeasts, they are used to produce ethanol-reduced wines. Contreras et al. (2014) reported *M. pulcherrima* effectively reduce the ethanol content when used sequential with *S. cerevisiae* to ferment Shiraz and Chardonnay grapes compared to a pure fermentation with *S. cerevisiae*. Quirós et al. (2014) suggested ethanol reduction to be the result of the respiratory metabolism of *M. pulcherrima*. In other words, in the presence of oxygen, *M. pulcherrima* would use respiration metabolism to derivate glucose into energy, whereas typically *S. cerevisiae* use fermentation even in an aerobic environment (Gonzalez et al., 2013). Indeed, Morales et al. (2015) studied the effects of aeration on both pure *S. cerevisiae* fermentation and simultaneous fermentation with *M. pulcherrima* and *S. cerevisiae* (mixing ratio 9:1). They observed a higher ethanol concentration reduction with a higher aeration of the must with both fermentation types, but the reduction was higher in the simultaneous fermentation.

Table 5. The main effects of pure *Metschnikowia* fermentation and sequential or simultaneous fermentations with *S. cerevisiae* compared to *S. cerevisiae* fermentation.

Ref.	Yeast	Strain	Raw material	Main effects*
Liu et al. (2020)	*M. pulcherrima*	70321	Bilberry	Ethanol ↓ Higher alcohols ↓ Esters ↑ Aldehydes ↑ Fatty acids ↑ Benzenes ↑
Sun et al. (2014)	*M. pulcherrima*	MJS22	Cherry	pH ↑ Titratable acidity ↑ Esters ↓ Higher alcohols ↓ Fatty acids ↓
	M. pulcherrima SeqF			pH ↑ Titratable acidity ↓ Esters ↑ Higher alcohols ↑ Fatty acids ↑
Boscaino et al. (2019)	*M. fructicola* SimF	AGYP28	Aglianico grape	Titratable acidity ↓ Total esters ↑ Higher alcohols ↑ Acetic acid ↓ Fatty acids ↑ Terpenes ↓

*compared to *S. cerevisiae* fermentation; ↑ increase in content; ↓ decrease in content; SimF simultaneous fermentation with S. cerevisiae; TA titratable acidity; seq. sequential fermentation with *S. cerevisiae*

The primary differences in wine yeasts are between the species (Tables 3–5), but notable differences are also observable between the yeast strains. The strain specific traits are related to the enzyme and metabolite secretions, affecting the primary and secondary aroma development of wines. In addition, the contribution of the levels of glycerol, mannoproteins, and volatile acidity and yeast to the stability of the wine colour differs from strain to strain. Most likely two yeast strains do not result in a similar wine even in the same environmental conditions. (Belda et al., 2016; Bely et al., 2008; Ciani et al., 2010; Jackson, 2000; Quirós et al., 2014) Sun et al. (2014) observed that two S. cerevisiae yeasts (D254 and EC1118) produced cherry wines with the different sensory properties. The wines had some similarities in the sensory properties, such as an intensive fruity odour and a moderate floral odour, but the D254 fermented wine had a more intensive *sweet* odour and less of a *green* odour compared to the EC1118 fermentation. Liu et al. (2018) studied, among other things, the effects of two *T. delbrueckii* strains on the chemical composition of bilberry wine. The resulting wines were different from each other. They had significantly different level of ethanol, residual sugars, organic acids, higher alcohols, total anthocyanins and vitisins. Barbosa et al. (2018) evaluated the

genetic and phenotypic differences of 65 *M. pulcherrima* strains from the Douro wine region. They did not find significant correlation between the genotypic profiles, neither as regards the winery or the geographic origin. However, they observed some significant differences between the studied strains. For example, 62 out of 65 strains tolerated at least 6 % (v/v) of ethanol, whereas remaining strains tolerate at least a 9 % ethanol concentration. This result also differs from other studies, which have reported M. pulcherrima to tolerate 3–5 % (v/v) ethanol (Comitini et al., 2011; Contreras et al., 2014; Morales et al., 2015). The other significant difference between the strains was in the β-lyase activity: all strains performed at least some β-lyase activity, but 17 strains out of 65 strains exhibited remarkable activity. The effect of β-lyase on the aroma complexity is through the release of volatile thiols from cysteinylated precursors (Roncoroni et al., 2011).

2.4.2 Effects of wine yeast fermentation on the colour of the beverage products

The studies of alcoholic fruit beverages are shown in Table 6 and discussed further in this chapter. In alcoholic beverages, as in every food product, colour is one of the most important sensory attributes. During the alcohol fermentation and the wine maturation, anthocyanins are condensed with the yeast metabolites, pyruvic acid and acetaldehyde, vinylphenols, or other flavonoids, forming more stable pyranoanthocyanins, polymeric pigments, and co-pigments, respectively. They generate more stable forms of anthocyanins to combat SO_2 bleaching, oxidative damage, and colour modification caused by the pH change. (Jackson, 2000; Suárez-Lepe and Morata, 2012) The colour of pyranoanthocyanins is usually reddish-brown or reddish-orange, and their typical maximal absorption wavelength is between 495–515 nm, being lower than those of the corresponding monomeric anthocyanins. (Morata et al., 2019)

Table 6. Examples of the effects of the wine yeast fermentations on berry and fruit raw materials.

Publication	Raw material	Yeasts	Vinification procedures	Analysed compounds	Effects on compounds important for sensory properties
Leino and Kallio (1993)	Black currant	*S. cerevisiae*	Commercial fermentation of juice and concentrate at winery.	Volatile compounds	Usage of juice or concentrate effected on the sensory properties. Two wines volatile compound profile had some differences. Main compounds were long chain fatty acids and ethyl esters. Content of monoterpenes decreased during the fermentations.
Czyzowska and Pogorzelski (2002)	Black currant Cherry	Two pectinases *S. cerevisiae,* Syrena	Hot maceration and/or enzymatic treatment for 2 h at 55 °C and pressing. Pre-fermentation: 5 % yeast starter to disinfected fruit. Fermentation: 0.5 L must per L of a wine pitching.	Total polyphenols, phenolic acids	Pectinase treatment increased total polyphenol contents of black currant and cherry wines. Hot maceration with enzymatic treatments had both increasing and decreasing effect on total polyphenols. Enzymatic treatments increased contents of some phenolic acids in both berries. Fermentation decreased phenolic acid contents in every case.
Czyżowska and Pogorzelski (2004)	Black currant Cherry	Two pectinases *S. cerevisiae,* Syrena	Hot maceration and/or enzymatic treatment for 2 h at 55 °C and pressing. Pre-fermentation: 5 % yeast starter to disinfected fruit. Fermentation: 0.5 L must per L of a wine pitching.	Monomeric anthocyanins and flavan-3-ols	Used enzyme effected on the extraction of anthocyanins. Heat maceration increased anthocyanin extraction of black currants with one enzyme. All fermentations decreased significantly anthocyanin contents. Heat maceration with enzymatic treatment decreased the flavan-3-ols in wines.

Publication	Raw material	Yeasts	Vinification procedures	Analysed compounds	Effects on compounds important for sensory properties
Duarte et al. (2010)	Raspberry	Fifteen *S. cerevisiae* strains were screened and a *S. bayanus* strain Two *S. cerevisiae* and a *S. bayanus* strain were studied further	Pre-grown yeast cell suspensions were adjusted to obtain an inoculum with 1.5 g/L. Fermentation at 22 °C without agitation.	Volatile compounds, sensory properties	Volatile compounds exhibited clear difference between yeasts. Fermentation with *S. bayanus* resulted significantly higher volatile sulphur compound contents than *S. cerevisiae*. Sensory evaluation of trained panel (n=12) resulted the highest and the lowest amount of aromatic descriptors to *S. cerevisiae* fermented beverages.
Čakar et al. (2018)	Black chokeberry Blueberry Raspberry Black berry Cherry	Two *S. cerevisiae*, ICV D254 and Lievito Secco A glycosidase	The must was supplemented with $K_2S_2O_5$ (0.1 g/kg). Fermentation without/with added sugar (°Brix set to 20.5) and/or glycosidase enzyme (EPG 100/g). Cherry wine also fermented without/with pit. Fermentation at 20 °C for 7-10 days. Storage at 12 °C for 6 months.	Monomeric flavan-3-ols, phenolic acids, ethanol content, pH, redox potential, anti-DPPH radical activity, TPC	No clear difference between yeast strains were observed. Additional sugar and glycosidase resulted the most abundant ethanol, monomeric phenolic compound contents, and TPC, but additional sugar had more effect. Pit in cherry wine fermentation increased phenolic acid contents, TPC, DPPH, and FRAP in every case.
Liu et al. (2017)	Mulberry	Tow *S. cerevisiae* strains Two *S. bayanus* strains Spontaneous fermentation	Adjusted sugar content, 0.05 % active dry yeast, fermentation temperature 18 °C	Alcohol, monomeric anthocyanins, TPC, TF; colour	Yeast species and strain effected on the contents of alcohol, total phenols, anthocyanins, and TF, and antioxidant activity. Spontaneous fermentation had more monomeric anthocyanins than inoculated fermentations.

Publication	Raw material	Yeasts	Vinification procedures	Analysed compounds	Effects on compounds important for sensory properties
Liu et al. (2018)	Bilberry	*S. cerevisiae* 1116 *T. delbrueckii* 291 and 70526 *Sz. pombe* 3796 and 705272	Juice freshly pressed and diluted 1:1 with water. pH adjusted to 3.5 and °Brix to 20.0. Pasteurisation at 95 °C for 5 min. Level of inoculations in every method: 10^7 CFU/mL. In the SeqF, *S. cerevisiae* inoculated at same level as first yeast at °Brix 10.0. In the SimF, *S. cerevisiae* and non-*Saccharomyces* inoculated at same time at same levels.	Higher alcohols, ethanol, pH, colour, glycerol, acetaldehyde, ethyl acetate, sugars, organic acids, monomeric anthocyanins, phenolic acids	SeqFs and SimFs were more different between each other than between PFs. PFs of yeast strains were different from each other. SimFs resulted with higher ethanol content, higher pH, more red and yellow colour, and lower contents of glycerol and acetaldehyde. non-*Saccharomyces* yeast PFs had significantly lower ethanol contents than *S. cerevisiae* PF. Residual sugars and sugar/acid ratio were generally higher in PFs.
Čakar et al. (2019)	Strawberry Apricot Peach Plum Sweet cherry	*S. cerevisiae* Lievito Secco	Fermentation without/with added sugar (°Brix set to 20.5), yeast added at the dose of 20 g 100 kg^{-1}. Drupe fruits were fermented without/with pits.	Phenolic acids, flavan-3-ols, flavonols, TPC, pH, TA, ethanol, °Brix,	The contents of phenolic compounds and antioxidant properties dependent on the fruit type and vinification procedure. Additional sugar increased ethanol content and enriched the phenolic contents. Pits in drupe fruit fermentations also increased the phenolic contents.
Lachowicz et al. (2019)	Apple	Four *S. cerevisiae* strains (SIHAFERM Pure Nature, SIHA rubino Cru, SIHA White Arome, SIHAFERM Finesse Red) Two *S. bayanus* (Lalvin C, Lalvin QA 23 YSEO)	The must was supplemented with $K_2S_2O_5$ (0.1 g/L) and nutrients (0.1 g/L). Yeasts were inoculated at rate 0.20 g/L. 1.0 g/L of *β*-CD was added to one sample. Fermentation at 20 °C for 3 weeks.	Phenolic acids, flavan-3-ols, monomeric anthocyanins, flavonols, ethanol, TA, organic acids, pH, colour (CIE), antioxidant activity, sensory evaluation	Fermentation of *S. cerevisiae* strains were more different between each other than *S. bayanus*. Use of *β*-CD significantly influenced increasing to bioactive compounds. Colour and antioxidant capacity after fermentation was depended on the yeast type and addition of *β*-CD. Fermentation with *β*-CD decreased all sensory properties.

Publication	Raw material	Yeasts	Vinification procedures	Analysed compounds	Effects on compounds important for sensory properties
Liu et al. (2020)	Bilberry	*S. cerevisiae 1116* *T. delbrueckii 291* *Sz. pombe 70572* *S. ludwigii 3447* *M. pulcherrima 70321* *L. thermotolerans 3434* *I. orientalis 3433* *H. uvarum 26650* *P. tannophilus 70352* *Z. bailii 70492*	10^7 CFU/mL inoculated to 50 mL sterilised juice, fermentation at 25 °C to dryness. Dynamic analysis of volatile compounds during the fermentations.	Volatile compounds, ethanol	Yeasts produced different content of ethanol in different rates. Fermentations with different yeast species resulted in different volatile profile. Volatile profiles changed over fermentation time.

Sz. *Schizosaccharomyces*; *L. Lachancea*; *I. Issatchenkia*; *H. Hanseniaspora*; *P. Pachysolen*; *Z. Zygosaccharomyces*; CD cyclodextrin; FB fermented beverage; FRAP Ferric Reducing Ability of Plasma test; DPPH 2,2-diphenyl-1-picrylhydrazyl; SimF simultaneous fermentation; PF pure fermentation; SeqF sequential fermentation; TA titratable acidity; TF total flavonoid content; TPC Total Phenolic Content; The CIE coordinates: L* lightness, a* red-green, b* yellow-blue, ΔE* the total colour difference

The co-pigmentation is a non-covalent interaction between the anthocyanin and other phenolic compounds in the fermentation solution. For example, phloroglunicol can act as a co-factor in the co-pigmentation. The co-pigmentation increases the absorbance and bathochromic shift of the anthocyanin solutions in the wine pH, because the colourless pseudobase of anthocyanins is the dominant form. In a low pH, the changes in the absorbance and bathochromic shift are only small. The chemical bond of the co-pigmentation is uncertain, but multiple explanations have been proposed. One explanation is the different electron densities between the aromatic rings: Anthocyanins are in a positively charged and electron poor flavylium form in wine, whereas other phenolic compounds are electron rich due to their nature as strong electron donors. This situation enables the charge transfer complexation. An alternative explanation is that the co-pigmentation interactions are hydrophobic, in which case planar aromatic molecules would arrange themselves in a π–π stacked manner. (Waterhouse et al., 2016)

The pyranoanthocyanins are formed by cycloaddition of nucleophilic substrate, such as pyruvic acid or acetaldehyde, to the C-4 position in the anthocyanin. The cyclisation forms an additional ring between the C-4 and the OH group at C-5 of the anthocyanin pyranic ring. (Quaglieri et al., 2017) Pyruvic acid and acetaldehyde are derived from sugars during the energy release. The energy metabolism of yeast is explained in Section 2.5. In addition, wine yeasts produce hydroxycinnamate decarboxylase (HCDC), which decarboxylates hydroxycinnamic acids formatting vinyphenols. This step is crucial in the formation of stable vinylphenolic pyranoanthocyanins due to the vinylphenols being condensed with anthocyanins. (Božič et al., 2020) The formation of the pyranoanthocyanins is a slow process. Typically, it takes more time than the sugar fermentation and it continues during the wine maturation. (Asenstorfer et al., 2003; Loira et al., 2015) Some pyranoanthocyanins, such as malvidin 3-*O*-glucoside pyruvic acid adduct (vitisin A), requires the presence of oxidants for formation (Asenstorfer et al., 2003). In addition, the concentration of pyruvic acid in the must typically first increases rapidly and then decreases, due to the reaction with the anthocyanins and the yeast ability to reabsorb or scavenge extracellular pyruvic acid. Non-*Saccharomyces* yeasts have been reported to improve the formation of stable anthocyanin pigments, such as pyranoanthocynins and polymeric pigments, due to their different microbial metabolism compared to *S. cerevisiae* (Božič et al., 2020; Escott et al., 2016).

Another phenomenon effecting the colour properties of the final wine is the anthocyanin adsorption of the yeast cell wall. Composition and porosity of the yeast cell wall differs by the species and strains. Morata et al. (2005) studied the anthocyanin adsorption of five *S. cerevisiae* strains. They noticed that the

strains differed in their rate of adsorption and the quality of the anthocyanins. The adsorption rate was between 1.60 and 5.85 % of the initial anthocyanin content. In their study, vitisins were weakly adsorbed and cinnamoyl derivatives of anthocyanins were strongly adsorbed. Božič et al. (2020) studied both *Saccharomyces* and non-*Saccharomyces* yeasts with high HCDC activity (>40 %). They observed that the adsorption rate of the anthocyanins and vinylphenolic pyranoanthocyanins depends on the yeast strain and species. In addition, they observed that the vinylphenolic pyranoanthocyanins were more readily adsorbed than the monomeric anthocyanins.

Interest towards the reduced alcohol content of wines has increased during the last decades. Health concerns, global climate change, growing market demand, and economical effects are the driving forces to research into the possibilities to reduce ethanol content. During the last 20 to 30 years, the ethanol content of wines has increased due to the global climate change. Warmer climates have increased the sugar content of mature grapes directly increasing the final ethanol content. Nowadays, consumers are more health-conscious and they are more aware of the health problems caused by high alcohol consumption. At the same time, consumers prefer well-structured and full-body wines, which are produced from grapes with an optimal phenolic maturity and, thus, increased sugar content. Higher sugar content has a linear correlation with a higher ethanol content. In addition, non-alcohol policies, e.g. for drivers and pregnant women, are advocated. Higher ethanol content also has an economical effect, as taxes are rated by alcohol content. (Ciani et al., 2016; Varela and Varela, 2019)

The effects of non-*Saccharomyces* yeasts on the ethanol content have been widely studied (Hu et al., 2020; Jiang et al., 2020; Sadineni et al., 2012). Varela et al. (2016) studied both simultaneous and sequential fermentation with *M. pulcherrima* or *S. bayanus* with *S. cerevisiae*. They observed significant ethanol reduction with both fermentation types compared to the pure *S. cerevisiae* fermentation. Furthermore, more significant ethanol reductions were observed with both sequential fermentations compared to the corresponding simultaneous fermentations. Similar results were reported by Belda et al. (2015): the sequential fermentation with *T. delbrueckii* and *S. cerevisiae* resulted significantly lower ethanol content compared to the pure *S. cerevisiae* fermentation. However, not all simultaneous or sequential fermentations with non-*Saccharomyces* yeast result in a reduced ethanol content. For example, Azzolini et al. (2015) studied the sequential fermentation with two commercial *T. delbrueckii* strains and *S. cerevisiae*. Only one sequential fermentation resulted in significant ethanol reduction compared to the pure *S. cerevisiae* fermentation. Similar results were reported by Sun et al. (2014). They studied pure and simultaneous fermentation of cherry juice with commercial *T.*

delbrueckii, *M. pulcherrima*, and two *S. cerevisiae* strains. They observed high ethanol tolerance (>10 %, v/v) for both non-*Saccharomyces* yeasts and the yeasts were able to finish the alcohol fermentation. In addition, they did not observe significant difference in the ethanol contents after pure or sequential fermentation with non-*Saccharomyces* yeasts compared to pure *S. cerevisiae* fermentation.

Use of non-*Saccharomyces* yeasts in pure or co-fermentation may have sensory quality improving characteristics (Morata et al., 2019b; Ramírez and Velázquez, 2018; Rêgo et al., 2020). Typically non-Saccharomyces yeast is secreting different enzymes, such as β-glucosidase, at different rates compared to *S. cerevisiae* (Fernández et al., 2000), thus effecting the volatile composition of the fermented products. Hu et al. (2020) studied the effects of simultaneous fermentation of *Hanseniaspora opuntiae*, *H. uvarum*, and *T.* d*elbrueckii* with *S. cerevisiae* on ponka (*Citrus poonensis*) citrus wine. All simultaneous fermentations improved the sensory properties of the ponka wine, but the wine fermented with *H. opuntiae* and *S. cerevisiae* resulted the best sensory properties of all wines (**Table 4**). They also reported the simultaneous fermentations to have a more complex aroma profile and higher concentrations of esters and higher alcohols, which resulted in improved sensory properties. Sadineni et al. (2012) reported similar results in their mango wine study. They used an *M. pulcherrima* strain and two *T. delbrueckii* strains in pure and simultaneous fermentations with *S. cerevisiae* (1:10 *S. cerevisiae*:non-*Saccharomyces*) to ferment mango juice. The simultaneous fermentations had higher contents of higher alcohols compared to their corresponding pure fermentations. The ethyl acetate concentration was higher after pure *M. pulcherrima* fermentation than its corresponding simultaneous fermentation, whereas every other simultaneous fermentation reduced the ethyl acetate content. In addition, Sadineni et al. observed that simultaneous fermentations had higher scores for *fruity* than the pure *S. cerevisiae* fermentation, and simultaneous fermentation with *M. pulcherrima* had the highest scores for *overall acceptability*, *colour*, and *taste*. Simultaneous fermentations with *T. delbrueckii* did not show any significant difference between each other. Other differences in the yeast metabolisms, such as the ability to use organic acids as an energy source, can have a major effect on the sensory properties of the fruit wines. In their study, Zhong et al. (2020) isolated and identified 23 fermentative yeasts with strong citric acid degradation ability from lemons, oranges, and soils. The most suitable yeast for kiwifruit (*Actinidia deliciosa*) fermentation was identified as *Pichia fermentans*. *P. fermentans* was able to significantly decrease the citric acid, tartaric acid, and malic acid contents, which reduced the flavour intensity of the organic acids.

2.5 Using cyclodextrins, enzymes and wine yeasts together in food processing

Cyclodextrins, commercial enzyme products, and wine yeast fermentations are all widely used alone in beverage productions. However, when used together these processes could have synergistic interactions between each other. The use of enzymes in wine making is already widely used and studied (Czyżowska and Pogorzelski, 2004; Ducasse et al., 2010; Egwim et al., 2013; Li et al., 2013). However, the effect of enzymes on fruit wines is less studied. During the pre-fermentation stage, enzymes are used to improve the juice yield, colour, and aroma extraction, and to increase sugars and polyphenols. Moreover, an additional pectinase enzyme may stabilise colour during ageing due to the increased extraction of anthocyanins and other polyphenols from grape skins (Ducasse et al., 2010). Guo et al. (2018) reported the use of the pre-fermentation pectinase and cellulase improved the growth of *T. delbrueckii* in jujube pulp by releasing ammonia and amino acids. Furthermore, enzymatic treatment significantly increased the total ester content, final ethanol level, and certain organic acids, such as lactic and pyruvic acid, and decreased acetic acid level. Finally, they observed significantly more aroma and body scores and improved colour properties in the enzyme treated jujube wine.

Even though many *S. cerevisiae* strains represent *β*-glucosidase activity (Hernández et al., 2003), the must can be enhanced with the additional *β*-glucosidase to improve the sensory properties. Li et al. (2013) studied the sensory properties and the volatile compounds after mango wine fermentation with or without pulp. The *β*-glucosidase was added to the fermentation vessel after 10 days of fermentation and an additional four days of incubation. The addition of the *β*-glucosidase significantly increased the glycerol, fructose, and free terpenol concentrations, but decreased the monoterpene hydrocarbons, which contribute to the fresh mango aroma. The content of fatty acids and their ethyl ester contents were also reduced. The enzyme maceration resulted in less *yeasty* and more *floral* and *fruity* taste properties compared to the non-macerated wines.

The post-fermentation enzyme procedures are part of clarification, filtration, and over-all microbial stabilisation. Kim and Park (2017) studied the post-fermentation enzymatic treatment with three *β*-glycosidases. They focused on the effects on the volatile compounds and the sensory properties of the black raspberry wines. All used enzymes significantly increased the total terpene, total ester, and total higher alcohol contents. They observed different sensory properties in the enzyme treated wines compared to the non-enzymatically treated wine. In addition, the impact of enzymatic treatments depends on the enzymes use. The most notable difference between the treatments was in the

off-odour intensity. All the enzymatic treatments caused higher intensity of fruity and floral aromas.

Cyclodextrins can be used to prevent phenolic compound degradation during the alcoholic fermentation. Lachowicz et al. (2019) studied the effect of the yeast strain, *β*-CD, and storage time (3 months at 4 ºC) on the physiochemical parameters, phenolic compound concentrations, sensory properties, and antioxidative activity of red apple cider. They observed that fermentation with the *β*-CD had a positive effect on the pH value, colour, antioxidative potency, and most of the polyphenols. However, fermentation with the *β*-CD resulted in red apple cider with the darkest colour. In addition, the addition of the *β*-CD resulted the highest anthocyanin concentration and increment in the phenolic acid and flavan-3-ol concentrations after 3 months storage, whereas the fermentation solely with the yeast decreased these contents.

Cyclodextrins are already used to preserve compounds and modify the sensory properties in food products. Enzymatic treatments can cause changes in the colour properties when anthocyanin aglycons are formed by the *β*-glucosidase activity. In addition, the enzymatic treatments can cause more intense sour, bitter, and astringents sensory properties due to the release of flavonoids, and a long incubation time can cause excessive evaporation of the aroma compounds. The CDs could act as protectors and flavour changers when added to the enzymatic treatment at different steps in the process: The CD treatment during the enzymatic treatment could prevent the colour changes and aroma release by protection of the anthocyanins and the volatile compounds, respectively. After enzymatic treatment, the use of a CD treatment could decrease the bitter and astringent properties of flavonoids and phenolic acids.

2.6 Concluding remarks

Bioprocesses and biopolymers are effective ways to change chemical and sensory properties of food products. However, many studies have shown that successful modification of food properties depends on the chemical properties of modification agent and food matrix used.

Cyclodextrins are an effective way to reduce undesirable taste and flavour properties in some food products. In addition, they can be used to improve the stability of many compound groups, such as anthocyanins and volatile compounds, and thus improve the retention of these compounds during processing. Encapsulation of hydrophobic compounds improves their water solubility, which has many beneficial effects, such as improved water solubility and an increase in bioavailability and antioxidative activity of the target compounds. In addition, it increases inhibitory activities against spoilage microorganisms. Studies have shown that successful use of the CD requires

good planning and understanding of the chemical composition of the food product.

Enzymatic treatments increase juice yield and have many beneficial effects on the technological properties of juices, such as improved pumping and filtering properties. However, enzymatic treatments have both negative and positive effect on the chemical and sensory properties of the juices by increasing the amounts of extracted compounds in the produced juice. Higher amounts of flavonoid compounds may increase the antioxidant activity and the colour intensity of the juice, but at the same time, these compounds may increase the bitter taste and astringent mouthfeel. In addition, enzyme products with high β-glucosidase activity tend to decrease the colour intensity of juices by the degradation of anthocyanins to their corresponding aglycones. Finally, enzymes with specific substrates, such as naringinase, can be used to decrease bitterness in food products.

Alcoholic fermentation comprehensively changes the physico-chemical properties of the raw material. During the fermentation, intra- or extracellular enzymes produced by the yeast degrade compounds, such as monomeric sugars, pectin, and proteins, and forms new ones, such as alcohols, fatty acids, and esters. *Saccharomyces cerevisiae* is the most utilised yeast in alcoholic fermentation. However, it lacks some properties, for example β-glucosidase activity, making other *Saccharomyces* or non-*Saccharomyces* yeasts desirable alternatives in wine making. In addition, new yeasts have shown properties, which answer the demands of consumers for more complex flavour and reduced ethanol content. Many studies have presented increased wine quality when *S. cerevisiae* is used in a simultaneous fermentation with non-*Saccharomyces* yeasts, such as *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, and *M. fructicola*. Use of non-*Saccharomyces* yeasts results in the more complex flavour properties by release of volatile aglycones from the glycoside forms. They also produce fewer undesirable compounds, such as free fatty acids, acetic acid, and higher alcohols, compared to fermentation with pure *S. cerevisiae*. Commonly, non-*Saccharomyces* yeasts are used in the simultaneous fermentation with *S. cerevisiae* due to their low ethanol tolerance. However, many fruits and berries contain less sugars than grapes making it possible to use non-*Saccharomyces* yeasts in the single yeast fermentation of fruit and berry wines.

The effects of the bioprocessing and biopolymer treatments on the chemical composition and sensory properties of fruits and berries are well studied and reported, but there is a lack of knowledge on the impact of the effect these kinds of processes have on lingonberries and black currants. The present thesis aims to fill this gap in the knowledge.

3 AIMS OF THE STUDY

The overall aim was to study the impact of bioprocessing methods on the compounds contributing to the sensory properties of lingonberry and black currant and the possibilities of modifying the sensory properties of products produced from these berries. Special focus was placed on characterisation of the changes in the non-volatile and volatile composition. The goal was to produce new knowledge about bioprocessing of Nordic berries and provide guidance for industrial juice processing and fermentation.

The first aim was to investigate the effects of *β*- and *γ*-cyclodextrins, gelatin, sequential gelatin and *β*-cyclodextrin, and NaOH treatments on the non-volatile compounds and sensory properties of commercial lingonberry (*Vaccinium vitis-idaea*) juice. (Study **I**)

The second aim was to study effect of the use of commercial enzyme products (pectinases, cellulase, and *β*-glucosidase) and heat treatments on the non-volatile and volatile composition of the Finnish lingonberries. (Study **II**)

The third aim was to determine the effects of *Saccharomyces* and non-*Saccharomyces* wine yeasts on non-volatile and volatile composition and sensory properties of products fermented from black currants (*Ribes nigrum*). (Study **III** and Study **IV**)

4 MATERIALS AND METHODS

4.1 Materials

4.1.1 Sample materials

Every juice and berry material used in this thesis were commercial products, which could have also been used in the industry level. The commercial lingonberry juice concentrate was purchased from Kiantama Ltd (Suomussalmi, Finland) and stored at -20 °C. The lingonberry juice concentrate was diluted 1:5.5 with ultrapure water (Milli-Q®, Millipore Corporation, Burlington, MA, US) or activated charcoal filtered water to obtain equivalent of 100% juice (**I**). The commercial frozen Finnish lingonberries (Artic International Ltd., Finland, **II**) and frozen Finnish black currants (Pakkasmarja Ltd, Suonenjoki, Finland, **III** & **IV**) were purchased from a local supermarket. The berries were from the same batch or the batches were pooled together.

4.2 Methods

4.2.1 Sample processing methods

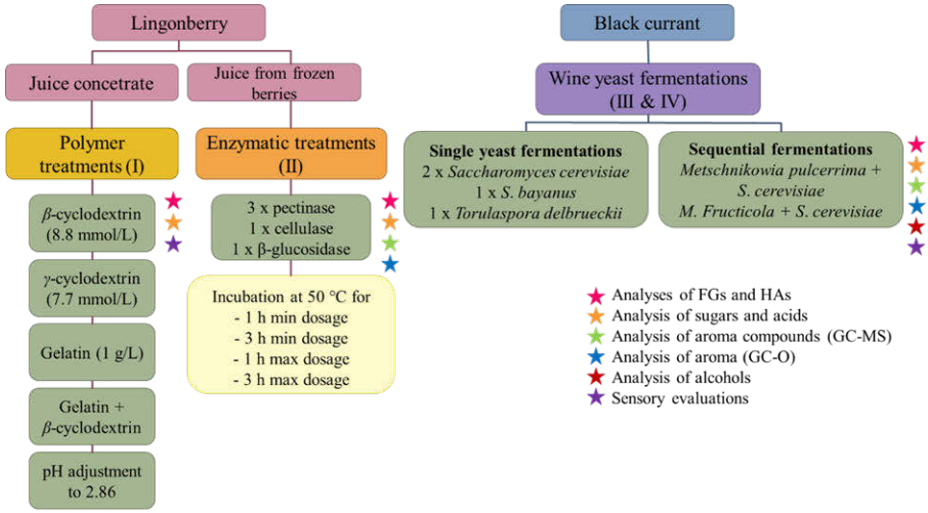

Figure 5. Sample preparations and chemical analyses in the studies **I**–**IV**.

Figure 5 summarises the procedures used in the sample preparation. Polymer treatments (**I**) included addition of 8.8 mmol/L of *β*-CD, 7.7 mmol/L of *γ*-CD, 1 g/L of gelatine or addition of gelatine and *β*-CD in the same levels as in the single polymer treatments. CDs and gelatine were added to juice and shaken vigorously. Gelatine treatment was left to +4 °C for 24 h. Then juice was

centrifuged at 6000g for 15 min and supernatant was collected. Optional addition of the *β*-CD was done after supernatant collection. In addition, pH of the lingonberry juice was adjusted adding 1.5 mol/L of sodium hydroxide (NaOH), which increased pH from 2.63 to 2.86.

The enzymatic treatments (**II**) were performed with two commercial and one non-commercial pectinase, one cellulase, and one *β*-glucosidase enzyme products. Treatments were performed at the constant temperature (50 °C) with changing incubation time (1 h or 3 h) and adding minimum or maximum dosage of the enzyme product recommended by the enzyme producers.

The wine yeast fermentations (**III** and **IV**) were performed using commercial wine yeasts, including two *S. cerevisiae* (Sc1 and Sc2), one *S. bayanus* (Sb), *T. delbrueckii* (Td), *M. pulcherrima* (M1), and *M. fructicola* (M2). For each fermentation, 0.25 g/L of rehydrated yeast was inoculated to the pasteurised black currant juice. In the sequential fermentations, *Metschnikowia* yeast was inoculated first, and after 24 h fermentation, *S. cerevisiae* yeast was inoculated. Both inoculations of sequential fermentations were at same rate as in the single yeast fermentations.

4.2.2 Sample preparation for determination with liquid chromatography (I–III)

Phenolic acids, flavonoid glycosides, and flavan-3-ols were extracted four times with ethyl acetate prior determination as described previously by Laaksonen et al. (2012). Briefly, 2–10 mL of each sample was measured and extracted four times with 5–10 mL of ethyl acetate. After ethyl acetate addition, sample was shaken vigorously for 1.5 min and centrifuged at 1000 g for 5 min. The supernatants were collected to the boiling flask and ethyl acetate were evaporated under vacuum at 35 °C to dryness. The compounds were re-dissolved to methanol and filtered with the 0.22 µm PTFE syringe filter.

Prior to the anthocyanin analysis, liquid samples were diluted 1:1 with acidified methanol (1:99, hydro chloride:methanol) and filtered with the 0.22 µm PTFE syringe filter.

4.2.3 Qualitative determination of flavonoid glycosides, flavan-3-ols, hydroxycinnamic acids, anthocyanins, and proanthocyanins (I–III)

Proanthocyanins (PAs) of the lingonberry juice were identified with HPLC-DAD-MS (**I**). Before chromatographic analysis, the PAs were purified from the lingonberry juice samples with activated Sephadex LH-20 column chromatography. A hydrophilic interaction chromatography methodology

(HILIC) was used to separate the PAs prior to the MS analysis. The purification of PAs, instrument parameters, eluents, and mobile phase gradients were described previously by Yang et al. (2016).

Identification of the flavonoid glycosides (FGs) and hydroxycinnamic acids (HAs) of the polymer treated lingonberry juice (**I**), and enzyme treated samples (**II**) and black currant juice and fermented beverages (**III**) were performed with an UHPLC-DAD-Q/Q or UHPLC-DAD-ESI-Q-TOF systems, respectively. Calibration of Q-TOF was achieved by injecting 10 mM sodium formate at 400 µL/min flow rate from a direct infusion syringe pump to the six-port valve for high-accuracy mass experiments in HPC mode. The flow gradient of the B elute used in the studies **I** and **II** was previously described by Mäkilä et al. (2016). It was modified to be suitable with UHPLC-column used in compound separation prior Q-TOF (**III**): 0−9.4 min, 2−18 %; 9.4−11.80 min, 18 %; 11.80−14.20 min, 18−20 %; 14.20−16.50 min, 20−60 %; 16.50−18.90 min, 60−2 %; and 18.9−21.20 min, 2 %. The compounds were identified with the help of reference compounds and literature (Lu et al., 2002; Mäkilä et al., 2016; Tian et al., 2019).

4.2.4 Quantitative determination of flavonoid glycosides, flavan-3.ols, hydroxycinnamic acids, anthocyanins, and procyanidins (I–III)

Quantitative determination of FGs, flavan-3-ols, HAs, and anthocyanins in the lingonberry juices (**I** and **II**) and black currant juice and fermented beverages (**III**) were performed with a UHPLC-DAD. Used elutes and chromatographic methods for FGs, HAs, and flavan-3-ols and for anthocyanins were described previously by Mäkilä et al. (2016) and Tian et al. (2017), respectively. Slight changes were made to the flow gradient of the B elute used in the anthocyanin analyses: 0–10 min, 5–10 %; 10–15 min, 10–15 %; 15–20 min, 15–40 %; 20–30 min, 40–90 %; and 30–35 min, 90–5 %. To quantify compounds, the five-point calibration curves were constructed with the commercial standard compounds.

The total PA contents of the polymer treated lingonberry juices (**I**) were determined spectrophotometrically as an equivalent of procyanidin B2 as previously described by Yang et al. (2016).

4.2.5 Determination of sugars and organic acids and ethanol (I–III)

Sugars and organic acids were determined as trimethylsilyl (TMS) derivatives from the lingonberry juices (**I** and **II**) and black currant juice and fermented beverages (**III**), using a GC-FID. Method was described previously by Ma et al.

(2017). Internal standards, sorbitol/xylitol and tartaric acid, and external standards, fructose, glucose, and sucrose and malic acid, citric acid, shikimic acid, quinic acid, and galacturonic acid, were used in the 5 g/L concentration and succinic acid in the 2.5 g/L to quantify sugars and organic acids.

Ethanol was determined from the fermented black currant beverages (**III**), using GC-FID as described previously by Liu et al. (2018). Internal standard was 2-butanol at the concentration of 5 %.

4.2.6 Determination of colour properties (III)

Colour properties were determined from the black currant juice and fermented beverages (**III**), using spectrophotometer as described previously by Liu et al. (2018). A plastic cell with a 1 cm optical pathway was used. The absorbances were recorded at 420 (yellow), 520 (red), and 620 nm (blue). Percentages of yellow (% yellow), red (% red), and blue (% blue) and the colour intensity (CI) and colour tonality (CT) were calculated as follow:

$$\text{Percentage of colour} = \frac{A_{420/520/620}}{CI}$$

$$CI = A_{420} + A_{520} + A_{620}$$

$$CT = \frac{A_{420}}{A_{520}}$$

4.2.7 Determination of volatile composition (II and IV)

The volatile composition of the enzyme treated lingonberry juice (**II**) and black currant juice and fermented beverages (**IV**) were determined with the GC-MS systems. All volatile extractions were carried out with divinylbenzene/ Carboxen/polydimethylsiloxane (DVB/CAR/PDMS 50/30 µm fiber, Supelco, Bellefonte, CA). Autosamplers were used to introduce fibre to the vial headspace. The temperatures of the thermal desorption were chosen not to exceed the column temperature limits. The identification of the volatile compounds was performed by probability-based matching of the obtained mass spectra with the mass spectra from the databases, such as NIST14 and Adams Essential Oil mass spectral library 2007, as well as from the literature (Iversen et al., 1998; Jung et al., 2017; Leino and Kallio, 1993; Y. Liu et al., 2018; Marsol-Vall et al., 2019b, 2018; Mikkelsen and Poll, 2002; Varming et al., 2006, 2004b; Wei et al., 2019). As a second criterion for the identification, linear temperature-programmed retention indices were calculated as Van den Dool & Kratz (**II**) or Linear retention (**IV**) indices (RI). Measured RIs were compared to data obtained from

authentic reference compounds or the literature and retention index databases. Internal standards were used to quantify the volatile compounds.

To determine the composition of the lingonberry juice (**II**), the GC-MS analyses were performed with a capillary intermediate polar column. For quantitative analyses, the samples were spiked with 4-methyl-2-pentanol as an internal standard. Mass spectra were recorded in electron impact (EI) mode at 70 eV within the mass range *m*/*z* 40–300. The transfer line and the ionisation source were thermostat at 225 and 210 °C, respectively.

The volatile compound separation of the black currant juice and fermented beverages (**IV**) were performed with non-polar and polar columns. The column oven was cool down with liquid nitrogen to -10 °C prior the analysis with non-polar column. With both columns, the mass selective detection was performed in the scan mode (35–350 *m*/*z*; EI (70 eV)). Interface temperature was set to 280 °C, and ion source to 200 °C. Quantitation of compounds was performed with the compound areas from analyses with the non-polar column. Internal standard was 2-octanol (50 ng/100 µL). Concentrations were calculated by dividing the compound area with the internal standard area.

4.2.8 Determination of aroma with GC-O (II and IV)

Aroma of the enzyme treated lingonberry juices (**II**), black currant juice, and *S. bayanus* and *T. delbrueckii* fermented beverages (**IV**) were determined with GC-FID coupled with an olfactory detector port (O) connected to an air humidifier. Eluent was split 1:1 from the column to FID and O. Same type columns were used and RIs were calculated with the same methods as in the GC-MS analyses. In the Studies **II** and **IV**, panellists (n=3 and n=5, respectively) were instructed to press button when odour was detected and hold until the end of the odour and then provide a description of the smell. GC-O evaluations were performed with untreated lingonberry juice and a lingonberry juice treated with *β*-glucosidase at maximum dosage for three hours. Qualitative data from GC-O were employed to evaluate which of the compounds identified in HS-SPME profiling made greater contributions to the overall aroma of enzymatically treated lingonberry juices. Only compounds for which all the evaluators agreed on the description were considered as positively confirmed.

In the GC-O analysis of the black currant samples (**IV**), each panellists evaluated each sample twice. Nasal impact frequencies (NIFs) were summed with individual signals (NIF 100 % corresponding to all assessors detecting an odour at the same time) and square of NIF (SNIF) was calculated with followed equation:

Duration of NIF (s) (≥ 40 %) × (NIF %/100).

4.2.9 Sensory evaluations (I and IV)

Sensory evaluations were performed with polymeric treated lingonberry juices (**I**) and fermented black currant juices (**IV**). Both sensory evaluation studies were performed in the controlled laboratory conditions (ISO 8589). Panellists were instructed to drink water and chew a small piece of unsalted cracker between samples to clean their palates.

For the sensory evaluation of lingonberry juices (**I**), taste and astringent properties of the juices were evaluated by 40 untrained panellists (29 females, 11 males, age 19–63). The attributes were defined with written examples and descriptions. The samples were presented (portion of 20 mL in 50 mL transparent glass beaker) in a partly fixed order: the untreated was the first sample followed by the treated juices in randomised order. First, the panellists were asked to evaluate attributes from the untreated juice (labelled R) on a scale of 0–10 and then the treated juice samples were compared to R using a five-point category scale (1=notably less attribute; 3=no difference; 5=notably more attribute). Finally, the panelists were asked to pick the most and the least pleasant samples. The evaluation was carried out using Compusense-five version 5.6 (Compusense Inc., Guelph, ON, Canada).

The descriptive analysis of the fermented black currant beverages (**IV**) was performed with 11 voluntary assessors (2 men, 9 women, age 20-59). In the first training session, assessors were subjected to basic taste test testing their ability to recognise tastes from ASTM standardised test solution concentrations.

The training for the generic descriptive analysis consisted of four one-hour sessions. In the first session, assessors were presented with three fermented beverage samples and were asked to describe their appearance, odour, taste, flavour, and texture followed with discussion. In further sessions, the lexicon were clarified by elimination of unnecessary attributes. In addition, the reference samples and their intensities were agreed on. All samples were presented to assessors at least once during the training sessions. The final profile had 12 attributes: 5 odour, 1 texture, 1 flavour, and 5 taste attributes (**Table 7**).

All samples were evaluated in triplicate during three sessions. 10 mL of beverages were served in covered tulip wine glass. The presentation order was randomised both among assessors and between sessions. Between the samples were one-minute break, when the assessors were instructed to clean their palate by drinking water and by chewing a piece of cracker. The intensities of sensory attributes were rated with a line scale (0= no attribute, 10= very strong attribute). Reference samples were anchored on the scale. Data was collected with Compusense Cloud version 20.0.

Table 7. Evaluated attributes, descriptions, and reference samples in the sensory evaluations.

Attribute	Description	Reference: concentration and manufacturer	Serving size and type	Intensity
Odour				
Total intensity of odour	Perceived overall intensity of odour			
Black currant	Odour typical for black currant	Mashed black currant in the covered scent bottle (Pakkasmarja, Ltd., Suonenjoki, Finland)	Few mashed berries in the 25 mL bottle.	7
Sweet	Sweet odour	Commercial whole black currant juice (Aten Marja, Ltd., Hietanen, Finland)	20 ml of the solution in a 50 ml transparent beaker	7
Sour	Sour odour			
Musty	Musty odour			
Mouthfeel[a]				
Viscosity	Viscous mouthfeel	Organic berry smoothie (Valio, Ltd., Pitäjänmäki, Finland)	20 ml of the solution in a 50 ml transparent beaker	6
Astringent	Puckering mouthfeel	0.10 % and 0.20 % $ALSO_4$ (Merck, Darmstadt, Germany)	20 ml of the solution in a 50 ml transparent beaker	2, 7
Orosensory				
Total intensity of flavour	Perceived overall intensity of flavour			
Black currant	Flavour typical for black currant	Commercial whole black currant juice (Aten Marja, Ltd., Hietanen, Finland)	20 ml of the solution in a 50 ml transparent beaker	8
Sweetness	Sweet taste	2 % sucrose solution (Alfa Aesar, Karlsruhe, Germany)	20 ml of the solution in a 50 ml transparent beaker	5
Bitterness	Bitter taste	0.07 % and 0.14 % caffein (Alfa Aesar, Karlsruhe, Germany)	20 ml of the solution in a 50 ml transparent beaker	3, 7
Sourness	Sour taste	0.14 % citric acid solution (Alfa Aesar, Karlsruhe, Germany)	20 ml of the solution in a 50 ml transparent beaker	5

[a] All mouthfeel and orosensory reference solutions were prepared in active-carbon filtered water.

4.2.10 Statistical analyses (I–IV)

In each study, statistical analyses were carried out with SPSS 25.0.0.1. (IBM SPSS Statistics Inc., Chicago, IL) and principal component analyses (PCA, **I-IV**) and partial least squares regression (PLS, **IV**) were constructed with Unscrambler X (versions 10 and 11, CAMO Inc, Oslo, Norway). One-way ANOVA with Tukey's test (**I-IV**), Tamhane's test (**I**), or LSD test (**IV**, sensory data), three-way ANOVA (**II, IV**), and independent sample t-test (**III** and **IV**) were used to determine statistical difference between the samples. The sensory data (I) from the five-point category scale was transformed to 0/1 data and analysed using McNemar's test.

Three-way ANOVA was used to determine the main effects of the enzyme product, enzyme dosage, incubation time, and the interactions between these changing parameters on the chemical composition of the freshly pressed lingonberry juice (**II**). In addition, three-way ANOVA was used to determine session, sample, and assessor effects on the results of the sensory evaluations of fermented black currant beverages (**IV**). The performance of the sensory panel was evaluated using PanelCheck software with Tucker-1 PCA and p-MSE plots resulting all assessors data included to data analysi

5 RESULTS AND DISCUSSION

5.1 Identification of flavonoid glycosides, flavan-3-ols, hydroxycinnamic acids, anthocyanins, and procyanidins (I–III)

Eleven phenolic compounds were identified in commercial lingonberry juice, lingonberries and black currants. The number of phenolic compounds were slightly different in these two berries: 58 compounds were identified in the lingonberries and 52 in the black currants (**Table 8**). However, the black currants used in the Study **III** contained approx. 135 mg/L of phenolic compounds, which is a notably higher amount compared to the phenolic content in the lingonberries used in the Studies **I** and **II** (approx. 80 mg/L).

Table 8. Non-volatile compounds identified from the commercial (**I**) or enzymatic treated lingonberry juice (**II**) and black currant juice and fermented black currant beverages (**III**).

			$[M+H]^+/[M-H]^-$ (*m/z*)			
No.	**Compound**	**UV λ_{max} (nm)**	**Nominal masses**	**Fragments[b]**	**Study**	**Ref.**
	Phenolic acid derivatives					
P1	4-*O*-caffeoylglucoside	215, 328	-/341	161, 133	**III**	7
P2	*p*-coumaric acid-4-*O*-glucoside	314	349/325	307, 291, 165/-	**II, III**	7
P3	Caffeic acid hexoside	313	343/341	-/-	**I**	1
P4	*E*-caffeoylglucose	216, 328	-/341	-/161, 133, 683	**III**	7
P5	3-*O*-caffeoylquinic acid*	319	355/-	-/-	**I, II**	1, 3
P6	5-*O*-caffeoylquinic acid	284, 322	-/353	-/ 191, 173[a]	**II**	7
P7	*p*-coumaroylquinic acid 1	208, 311	339/337	147, 119/163[a]	**III**	7
P8/P8.2	*p*-coumaric acid *O*-glucoside	210, 311	-/325	-/145[a]	**III**	7
P9	1-*O*-Benzoyl-beta-glucose	275	285	569/-	**I**	1
P10	Vanillic acid hexoside	323	-/329	-/-	**I**	1
P11	Caffeic acid	217, 323	181/179	-/161, 339	**III**	1, 7
P12	Ferulic acid hexoside	326	357/355	195/193, 171[a]	**I, II, III**	1, 5
P13	*p*-coumaric acid*	310	165/163	-/-	**I, II, III**	1, 2
P14	Ferulic acid hexoside	320	357/355	195/-	**I, II, III**	1
P15	*E*-ferulic acid*	295,322	195/193	-/-	**II**	**7**
P16	2"-*O*-caffeoylarbutin	322	435/-	325/-	**I, II**	4, 6, 5
P17	Ferulic acid hexoside	313	357/355	195/192	**I, II**	1, 5
P18	*p*-coumaroylmonotropein	286, 311	-/535	-/453,433	**II**	**7**
P19	Ferulic acid derivative	325	-/387	-/193	**I, II**	1, 2
P20	*E*-caffeoyloxymethylene-glucosyloxybutenenitrile	213, 330	-/436	-/-	**III**	7
P21	*E*-coumaroyloxymethylene-glucosyloxybutenenitrile	210, 313	422/420	-/163, 119	**III**	7
P22	*Z*-coumaroyloxymethylene-glucosyloxybutenenitrile	216, 310	422/420	-/163	**III**	7
P23	*E*-feruloyloxymethylene-glucosyloxybutenenitrile	218, 327	452/450	-/193	**III**	7
P24	*E*-*p*-coumaroyloxymethylene-oxybutenenitrile[c]	217, 313	-/258	-/163, 184, 214	**III**	7
P25	*Z*-coumaroyloxymethylene-oxybutenenitrile[c]	219, 310	-/259	-/163, 214	**III**	7
	Flavonols					
F1	Myricetin 3-*O*-rutinoside	210, 356	627/625	319, 481/-	**III**	1, 7
F2	Myricetin 3-*O*-glucoside	210, 355	481/-	319/-	**III**	1, 7
F3	Myricetin 3-*O*-arabinoside	216, 355	451/-	319/-	**III**	7
F4	Myricetin 3-*O*-malonyl-galactoside	208, 254	567/565	319/521	**III**	7
F5	Myricetin (aglycone)	219, 371		-/-	**III**	7
F6	Quercetin 3-*O*-rutinoside	205,352	611/-	465, 303[a]/-	**I, III**	1, 4, 5
F7	Quercetin 3-*O*-galactoside	354	465/-	-/-	**I, II**	1, 4, 5
F8	Quercetin 3-*O*-glucuronide*	351	479/477	-/-	**I**	1, 4
F9	Quercetin 3-*O*-glucoside*	205, 354	465/463	417, 303[a]/-	**I, II, III**	1, 4, 5
F10	Quercetin 3-*O*-xyloside	354	435/433	-/-	**I, II**	1, 4, 5
F11	Quercetin 3-*O*-arabinoside	354	435/433	-/-	**I, II**	1, 4, 5
F12	Quercetin 3-*O*-arabinofuranoside	354	435/434	-/-	**I, II**	1, 4, 5
F13	Quercetin 3-*O*-rhamnoside*	346	449/448	-/-	**I, II**	1, 4, 5
F14	Quercetin 3-*O*-4"-(3-hydroxy-3-methylglutaroyl)-rhamnoside	346	593/591	-/-	**I, II**	1, 4, 5
F15	Quercetin-3-O-(6″-malonyl)-glucoside	218, 352	551/550	303/-	**III**	7
F16	Quercetine (aglycone)	368	303/301		**I, II**	1, 2, 4
F17	Kaempferol rutinoside	208, 350	595/593	449, 287/284[a]	**III**	7
F18	Kaempferol galactoside	210, 343	449/-	287/-	**III**	7
F19	Kaempferol-(3-hydroxy-3-	263, 344	577/575	287/-	**II**	1

No.	Compound	UV λ_{max} (nm)	$[M+H]^+/[M-H]^-$ (*m/z*) Nominal masses	Fragments[b]	Study	Ref.
	methylglutaroyl)-rhamnoside					
F20	Quercetin derivative	266, 357	569/567	207/223, 279	**II**	1
F21	Quercetin 3-*O*-(6"-benzoyl)-galactoside	355	569/567	303/-	**I, II**	1
F22	Quercetin	220, 368	303/302		**III**	7
		Anthocyanins				
A1	Delphinid 3-*O*-glucoside	277, 523	465/-		**III**	7, 8
A2	Delphinid 3-*O*-rutinoside	277, 524	611/-		**III**	7, 9
A3	Cyanidin 3-*O*-galactoside	520	449/-	287/-	**I, II**	7, 10
A4	Cyanidin 3-*O*-glucoside	279, 514	449/-		**I, II, III**	7, 10
A5	Cyanidin-3-*O*-arabinoside	520	419/-	287/-	**I, II**	7, 10
A6	Cyanidin 3-*O*-rutinoside	280, 516	595/-		**III**	7
A7	Petunidin 3-*O*-rutinoside	526, 277	625/-	433/-	**III**	7
A8	Peonidin 3-*O*-rutinoside	273, 515	609/-		**III**	7
A9	Delphinidin-3-*O*-coumaroyl-glucoside	281, 528	611/-	303/-	**III**	7
A10	Delphinidin (aglycone)	267, 505			**III**	7
A11	Cyanidin-3-*O*-coumaroyl-glucoside	281, 523	595/-	287/-	**III**	7
A12	Cyanidin (aglycone)	278, 503			**III**	7
		Pyranoanthocyanins				
A13	Cyanidin rutinoside pyruvic acid[c]	N/A	663/-	595/-	**III**	9
A14	Cyanidin rutinoside acetaldehyde[c]	N/A	619/-	595, 473/-	**III**	9
		Flavan-3-ols				
FL1	Epi-gallocatechin	210, 287	307/305	-/611	**I, III**	1
FL2	Catechin	202, 283	-/289	-/465	**I, II, III**	1
FL3	Epi-catechin	204, 279	291/-	-/-	**II, III**	1
FL4	B-procyanidin dimer 1	278	579/577	291/289	**I, II**	1
FL5	B-procyanidin dimer 2	279	579/577	291/289	**II, III**	1
FL6	B-procyanidin dimer 3	279	579/577	291/289	**II**	1
FL7	A-procyanidin trimer 1	278	865/863	577/575	**I, II**	1
FL8	A-procyanidin trimer 2	279	865/863	577/575	**I, II**	1
FL9	A-procyanidin dimer	279	-/577	-/289	**I, II**	1
		Others				
C1	Citric acid methyl ester	195, 294	-/205	-/191, 111, 143	**III**	7

* Identification with external standard compound; [a] MS/MS results with Q-TOF; [b] Ion source fragments (**I**) or collision cell fragments (**II** and **IV**); [c] Identified only from fermented beverages; 1 Tian et al. (2017); 2 Seraglio et al. (2018); 3 da Silveira et al. (2017); 4 Hokkanen et al. (2009); 5 Ek et al. (2006); 6 Ieri et al. (2013); 7 Mäkilä et al. (2016); 8 Aneta et al. (2013); 9 Villiers et al. (2004); 10 Lee & Finn (2012).

A total of 58 non-volatile compounds (14 phenolic acids or their derivatives, 8 flavonol glycosides, 3 acylated flavonol glycosides, quercetin aglycone, 3 anthocyanins, 4 organic acids, and 2 sugars) were identified or tentatively identified from the lingonberry juices (**I** and **II**; **Table 8**) using the literature (da Silveira et al., 2017; Ek et al., 2006; Hokkanen et al., 2009; Ieri et al., 2013; Seraglio et al., 2018; Tian et al., 2017) and external standard compounds. In addition, twenty-four procyanidins were identified in the lingonberry juices with HILIC-UHPLC-DAD-MS system (**Table 9**; **I**). PAs of A- and B-type with a degree of polymerisation (DP) of 1–9 were identified. A- and B-type PAs with the DP values of 2, 3, 4, 5, and 9 were identified. DP values of 6, 7, and 8 were identified only with B type PAs.

Table 9. Procyanidins identified from the lingonberry juice and their MS data (**I**).

		Number and type of subunits			Detected mass		Type of linkage
Peak#	**DP**	**(E)C**	**(E)GC**	**Molecule mass**	**$[M-H]^-$ (*m/z*)**	**$[M-2H]^{2-}$ (*m/z*)**	
1	1	1	0	289.1	289.5		-
2	1	0	1	305.1	305		-
3a	2	2	0	578.1	577.6		B
3b	2	2	0		575.6		A
4a	2	1	1	594.1	593.1		B
4b	2	1	1		591.6		A
5	3	0	2	610.1	609.7		B
6a	3	3	0	866.2	865.7		B
6b	3	3	0		863.7		A
7	3	2	1	882.20	879.9		A
8a	4	4	0	1154.2	1153.7		B
8b	4	4	0		1151.7		A
9a	4	3	1	1170.3	1169.2		B
9b	4	3	1		1168.7		A
10a	5	5	0	1445.4	1443.6		B
10b	5	5	0		1441.3		A
11a	5	4	1	1461.4	1459.8		B
11b	5	4	1		1457.0		A
13	7	4	3	2066.4		1032.8	
14	7	3	4	2082.4		1041.6	
15	9	7	2	2626.6		1314.7	
16a	9	6	3	2642.6	2640.8		B
16b	9	6	3		2637.5		A
17	9	5	4	2658.6		1329.5	

Abbreviations: DP degree of polymerisation, (E)C (epi)catechin, (E)GC gallocatechin

The identified phenolic acids and their derivatives differend slightly between the lingonberry juices studied. In the diluted lingonberry juice concentrate (**I**), 10 phenolic acids and their derivatives were identified, whereas 11 were identified in the enzymatic treated lingonberries. Seven of these phenolic acids and derivatives were identified in both lingonberry juice types (**Table 8**). This difference may be the result of different raw material types, even though both were Finnish lingonberries or juice made from them: in the Study **I**, commercial lingonberry juice concentrate was diluted to correspond to 100 % juice, whereas frozen whole lingonberries were used to produce the juice in the Study **II**. Some difference can be caused by the seasonal and terroir differences, such as weather conditions (Bujor et al., 2018) and growth latitude (Zheng et al., 2012). Processing of lingonberries into the concentrated juice is probably affected by the phenolic composition (Elik et al., 2016; Pap et al., 2010).

Another issue having an effect concerns the analytical instruments used in the different studies. In the Study **II**, the Q-TOF used has a higher resolution and mass accuracy compared to the tandem quadrupole used in the Study **I** (**Table 8**; Allen and McWhinney, 2019)

Galacturonic acid was determined only from the commercial lingonberry juice used in the Study **I**. Typically, free galacturonic acid does not appear in the berries or fruits. It is a sugar acid and the main monomeric unit in pectin, released by pectinolytic enzymes, which are used in the food industry to increase the juice yield (Jayani et al., 2005). Quercetin glucuronide has not previously been reported in lingonberry juice and was only identified in the Study **I**. Study **II** reported the identification of kaempferol-(3-hydroxy-3-methylglutaroyl)-rhamnoside, but no kaempferols were identified in the Study **I**. Kaempferol glycosides have been reported previously in lingonberries by Antolak et al., (2017), Ek et al. (2006), and Lehtonen et al. (2010).

A total of 52 non-volatile compounds (14 phenolic acids or their derivatives, 7 flavonol glycosides, 2 acylated flavonol glycosides, 2 flavonol aglycones, 3 flavan-3-ols, 6 anthocyanins, 2 acylated anthocyanins, 2 pyranoanthocyanins, 2 anthocyanin aglycones, 7 organic acids, 2 citric acid methyl esters, and 3 sugars) were identified or tentatively identified in the black currant juice and FBs (**III**; **Table 8**). The PAs were identified as derivatives of caffeic, p-coumaric, and ferulic acids, in addition to free caffeic acid and *p*-coumaric acid. The flavonols were identified as glycosides of myricetin, quercetin, and kaempferol, and the anthocyanins were identified as glycosides of delphinidin, cyanidin, and peonidin. Trace amounts of the pyranoanthocyanins were detected only after fermentation in the black currant juice.

Four phenolic acid compounds containing a nitrile group, namely *E*-caffeoyloxymethyleneglucosyloxybutenenitrile (compound 21 in **Table 8**), *E*-coumaroyloxymethyleneglucosyloxybutenenitrile (22), *Z*-coumaroyloxymethylene-glucosyloxybutenenitrile (23), and *E*-feruloyloxymethyleneglucosyloxybutenenitrile (24), have previously been identified in black currant juice by Mäkilä et al. (2016). In addition, two new phenolic acid compounds were detected but only in the fermented samples compared to non-fermented black currant juice. They were tentatively identified based on the mass spectra as aglycones of compounds 22 and 23, respectively, namely, *E*-coumaroyloxymethyleneoxybutenenitrile (25) and *Z*-coumaroyloxymethyleneoxy-butenenitrile (26). An odd-number nominal mass [M] of *m*/*z* 259 indicated the presence of odd numbered nitrogen atoms in the molecules. The fragmentation pattern with both ionisation modes (m/z^+ 96, 114, 147, 242 [M+H-H_2O], m/z^- 117, 163 [M-H-coumaric acid]) were similar to compounds 22 and 23. These compounds are probably the result of the glucosidase activity of yeasts. Even though the content of the nitrile containing compounds 21 and 24 were lower after fermentation, corresponding aglycones of these compounds were not detected.

Two pyranoanthocyanins (compounds 60 and 61 in **Table 8**), were detected and tentatively identified in the fermented beverages using a Q-TOF mass spectrometer

(**III**), but not in the black currant juice. The mass of the parent ions were $[M+H]^+$ 663 and 619, respectively, and the daughter ion was $[M+H]^+$ 595 at both peaks indicating both compounds to be cyanidin-3-*O*-rutinoside derivatives. Furthermore, peak 60 was tentatively identified as cyanidin-3-*O*-rutinoside-pyruvic acid ($[M+H+\text{pyruvic acid}]^+$ 595+68) and peak 61 as cyanidin-3-*O*-rutinoside-acetaldehyde ($[M+H+\text{acetaldehyde}]^+$ 595+24).

5.2 Identification of the volatile composition of the enzymatic treated lingonberry juices (II), the black currant juice and the fermented beverages (IV)

A total of 97 aroma active compounds (8 acetates, 8 fatty acids, 21 higher alcohols, 13 aldehydes, 1 benzene, 16 esters, 1 ether, 4 ketones, 26 terpenes) were identified from the black currant juice (BCJ) and fermented beverages (FBs) (**Table 10**). Twenty-six aroma active compounds were detected only in the BCJ, and 38 only in the FBs, and 34 in both sample types.

A total of 24 volatile compounds (5 aldehydes, 2 alcohols, 5 ketones, 1 fatty acid, 3 hydrocarbons, 4 esters, and 4 terpenoids; **Table 10**) were identified in the enzymatic treated lingonberry juices (**II**). Among these compounds, six volatile compounds, 2,3-butanedione, 2-methylpropanoate, hexanal, eucalyptol, linalool, and methyl benzoate, were identified and described by mutual agreement of all three evaluators in the GC-O analysis.

Table 10. Volatile compounds identified from the black currant juice and fermented beverages, retention indices of non-polar and polar columns, compound occurrence in the sample types, and used identification methodologies (**IV**).

		RI* Rxi-5		RI* Zb-WAX		Occurrence			
No.	**Compound**	**Measured**	**Literature**	**Measured**	**Literature**	**Juice**	**Wine**	**Identification[a]**	**Ref.**
	Acetates								
V1	Methyl acetate	499	487			+	+	MS, RI	
V2	Ethyl acetate[b]	597	606			+	+	MS, RI, STD	6, 7
V3	Propyl acetate	709	708			+		MS, RI, STD	
V4	2-Methylpropyl acetate	773	776				+	MS, RI, STD	
V5	Butyl acetate	813	816				+	MS, RI, STD	4
V6	3-Methylbutyl acetate	876	876	1120	1117		+	MS, RI, STD	4
V7	2-Methylbutyl acetate	879	873				+	MS, RI, STD	7
V8	2-Phenylethyl acetate	1268	1259	1814	1812		+	MS, RI, STD	7
	Fatty acids								
V9	Acetic acid[b]	604	610	1468	1464		+	MS, RI, STD	1, 4, 7
V10	2-Methylpropanoic acid	748	758	1585	1585		+	MS, RI, STD	
V11	Butanoic acid	774	795	1647	1621		+	MS, RI, STD	3, 4, 7
V12	3-Methylbutanoic acid	830	831				+	MS, RI, STD	
V13	2-methylbutanoic acid	840	846				+	MS, RI, STD	
V14	Hexanoic acid	967	974	1864	1857		+	MS, RI, STD	3, 4, 7
V15	Octanoic acid	1162	1174	2074	2083		+	MS, RI, STD	3, 7
V16	Decanoic acid	1357	1373	2269	2272		+	MS, RI, STD	3
	Higher alcohols								
V17	2-Methyl-3-buten-2-ol	594	600	1041	1028	+	+	MS, RI	4
V18	2-Methyl-1-propanol	615	614	1110	1109		+	MS, RI, STD	4
V19	Butanol	652	656	1164	1152		+	MS, RI, STD	4
V20	3-Methyl-1-butanol	731	739			+	+	MS, RI, STD	1, 3
V21	2-Methyl-1-butanol	735	739				+	MS, RI, STD	1
V22	Pentanol	765	759	1255	1255	+	+	MS, RI, STD	4
V23	3-Methyl-2-buten-1-ol	776	773	1319	1318	+	+	MS, RI, STD	7
V24	2,3-Butanediol	790	790	1577	1554		+	MS, RI	1
V25	2-Furamethanol	857	885				+	MS, RI, STD	8
V26	*E*-2-hexenol	867	868			+		MS, RI, STD	4
V27	Hexanol	868	860	1350	1360	+	+	MS, RI, STD	4, 7
V28	Heptanol	972	982	1451	1443		+	MS, RI, STD	4
V29	3-(Methylthio)-1-propanol	981	982	1716	1714		+	MS, RI, STD	8
V30	2-Ethyl-1-hexanol	1030	1032	1486	1487		+	MS, RI, STD	4
V31	Octanol	1071	1068	1553	1558		+	MS, RI, STD	4, 7
V32	2-Phenethyl alcohol	1128	1118	1913	1918		+	MS, RI, STD	1, 7
V33	Nonanol	1172	1173	1663	1661		+	MS, RI, STD	4, 7
V34	Decanol	1274	1263	1758	1765		+	MS, RI, STD	7
V35	Dodecanol	1479	1475				+	MS, RI, STD	
V36	***Aldehydes***								
V37	3-Methylbutanal	642	645			+	+	MS, RI, STD	
	2-Methylbutanal	653	658	969	926	+	+	MS, RI, STD	
V38	Pentanal[b]	689	707			+		MS, RI, STD	
V39	*Z*-2-Pentenal	753	754			+		MS, RI, STD	4
V40	Hexanal[b]	800	807	1077	1076	+		MS, RI, STD	4, 5, 6
V41	Furfural	836	831	1464	1460	+	+	MS, RI	9
V42	Heptanal	902	906	1184	1188	+	+	MS, RI, STD	6
V43	*Z*-2-Heptenal	960	957	1316	1321	+		MS, RI, STD	4, 6
V44	Benzaldehyde	971	960	1523	1509	+	+	MS, RI, STD	
V45	Octanal[b]	1004	1000	1280	1281	+	+	MS, RI, STD	4, 6, 7
V46	*Z*-2-Octenal	1062	1049		1320	+		MS, RI, STD	4, 6, 7

		RI* Rxi-5		RI* Zb-WAX		Occurrence			
No.	**Compound**	**Measured**	**Literature**	**Measured**	**Literature**	**Juice**	**Wine**	**Identification[a]**	**Ref.**
V47	Nonanal	1107	1102	1386	1385	+	+	MS, RI, STD	1, 4, 5
V48	*Z*-2-Nonenal	1166	1163	1528	1527	+		MS, RI, STD	4
V49	***Benzenes***								
V50	1,3-Dimethylbenzene	877	907			+	+	MS, RI, STD	
	Vinylbenzene	898	883				+	MS, RI, STD	7
V51	***Esters***								
V52	Methyl propanoate	616	611			+		MS, RI	
	Ethyl propanoate	706	713				+	MS, RI, STD	1
V53	Methyl butanoate[b]	717	724	974	971	+	+	MS, RI, STD	1, 4, 6
V54	Ethyl 2-methylpropanoate	757	756				+	MS, RI, STD	
V55	Methyl 2-methylbutyrate	777	780	999	1004	+		MS, RI, STD	5
V56	Ethyl butanoate	800	802	1028	1036	+		MS, RI, STD	1, 4, 5
V57	Methyl hexanoate	924	924			+	+	MS, RI, STD	4, 6, 7
V58	Ethyl 3-hydroxybutanoate	935	912			+		MS, RI	4, 7
V59	Ethyl hexanoate	999	1002	1230	1232		+	MS, RI, STD	3, 6, 7
V60	Methyl octanoate	1123	1127	1383	1389	+		MS, RI, STD	1, 3, 4
V61	Ethyl octanoate	1195	1198	1433	1436		+	MS, RI, STD	3, 4, 6
V62	Methyl decanoate	1324	1326			+		MS, RI, STD	3, 4, 6
V63	Ethyl 9-decenoate	1388	1376				+	MS, RI	
V64	Ethyl decanoate	1390	1406	1635	1634		+	MS, RI	3, 4, 6
V65	3-Methylbutyl octanoate	1449	1442	1653	1658		+	MS, RI	3
V66	Ethyl dodecanoate	1595	1577	1839	1833		+	MS, RI, STD	3
V67	3-Methylbutyl pentadecanoate	1647	1647	1859	1864		+	MS, RI, STD	
V68	***Ethers***								
V69	3-Ethoxy-1-propanol	842	837	1381	1389		+	MS, RI	
	Ketones								
V70	2-Pentanone[b]	677	687			+	+	MS, RI, STD	
	1-Octen-3-one	979	981	1295	1310	+		MS, RI, STD	4, 6
V71	6-Methyl-5-hepten-2-one[b]	988	986	1332	1346	+		MS, RI, STD	7
V72	2-Octanone	992	994				+	MS, RI, STD	
V73	***Terpenes***								
V74	*α*-pinene[b]	946	948	1003	1032	+	+	MS, RI, STD	2, 4, 5
	Camphene	964	954	1051		+		MS, RI, STD	4, 5
V75	*β*-Myrcene	993	989	1152	1145	+		MS, RI, STD	4, 5, 6
V76	*α*-Terpinene	1009	1012	1173	1178	+		MS, RI, STD	2, 4, 6
V77	*α*-Terpinolene	1016	1017	1271	1274	+		MS, RI, STD	2, 4, 5
V78	*δ*-3-Carene	1024	1026	1141	1148	+		MS, RI, STD	4, 5, 6
V79	*o*-Cymene[b]	1036	1025	1260	1258	+	+	MS, RI, STD	2, 4, 5
V80	D-Limonene	1040	1025	1188	1202	+	+	MS, RI, STD	2, 4, 5
V81	1,8-cineole[b]	1045	1031	1200	1208	+	+	MS, RI, STD	2, 4, 5
V82	*γ*-Terpinene	1070	1062	1236	1238	+		MS, RI, STD	2, 4
V83	*E*-Linaloloxide	1085	1087	1428	1420	+	+	MS, RI	2, 7
V84	*p*-Cymenene	1103	1091	1431	1430	+	+	MS, RI	5
V85	Linalool[b]	1104	1100	1544	1537	+	+	MS, RI, STD	1, 2, 3
V86	Rose oxide	1120	1111	1347	1338	+	+	MS, RI, STD	2, 5, 7
V87	*α*-Campholenal	1144	1132	1484	1486	+		MS, RI	5
V88	4-Acetyl-1,4-dimethyl-1-cyclohexene	1170	1161	1512	1491	+	+	MS, RI	
V89	*p*-Cymen-8-ol	1195	1193			+		MS, RI	5
V90	Terpinen-4-ol	1198	1179	1596	1591	+	+	MS, RI, STD	1, 2, 4
V91	*α*-Terpineol	1210	1207	1694	1691	+		MS, RI, STD	10
V92	*δ*-Carvone	1266	1246	1724	1728	+		MS, RI, STD	7
V93	*E*-Linalool oxide acetate (pyranoid)	1296	1292	1622	1619	+	+	MS, RI	7

		RI* Rxi-5		RI* Zb-WAX		Occurrence			
No.	Compound	Measured	Literature	Measured	Literature	Juice	Wine	Identification[a]	Ref.
V94	Bornyl acetate	1307	1289	1565	1570	+	+	MS, RI, STD	2, 4, 5
V95	Citronellyl acetate	1355	1353			+		MS, RI	10
V96	*β*-Damascenone	1410	1440	1815	1813	+	+	MS, RI, STD	1, 2, 3
V97	*β*-Caryophyllene	1464	1464	1585	1596	+	+	MS, RI, STD	4, 5, 6
V98	*α*-Humulene	1498	1488	1659	1680	+	+	MS, RI, STD	6

* Linear retention indices; a Identification with MS mass spectra, RI matching retention index to literature, STD with internal standard; b identified in the lingonberry; 1 Varming et al. (2004); 2 Varming et al. (2006); 3 Leino & Kallio (1993); 4 Jung et al. (2017); 5 Marsol-Vall et al. (2018); 6 Iversen et al. (1998); 7 Liu et al. (2018); 8 Wei et al. (2019); 9 Mikkelsen & Poll (2002); 10 Marsol-Vall et al. (2019b)

5.3 The influences of bioprocessing

5.3.1 Effects of polymeric and NaOH treatments on lingonberry juice (I)

Generally, the gelatine treatment of the commercial lingonberry juice had the most significant decreasing effect on the phenolic compound contents addition of *β*-CD slightly increased the contents. Treatment with gelatine and *β*-CD did not result in a similar juice as the gelatine treatment. Figure 6 represents the total amounts of phenolic compounds in all the polymer and NaOH treated lingonberry samples.

The polymer and NaOH (increased pH) treatments did not significantly affect the anthocyanin contents compared to the untreated lingonberry juice, but the CD treatments had a significantly higher anthocyanin content compared to other treatments. This phenomenon can be explained by the complexation of CDs and anthocyanins, which may protect anthocyanins during the storage and sample preparation steps from the induced degradation (Fernandes et al., 2018; Howard et al., 2013). No significant difference of the total phenolic acid contents was detected between the polymer or NaOH treated lingonberry juices compared to untreated juice. Nonetheless, the use of *γ*-CD slightly increased the phenolic acid content compared to the untreated lingonberry juice. Gelatine treatment resulted in the lowest level of total phenolic acid content. The phenolic acid contents were marked lower than a previously reported contents by Grace et al. (2014) and Hellström et al. (2009).

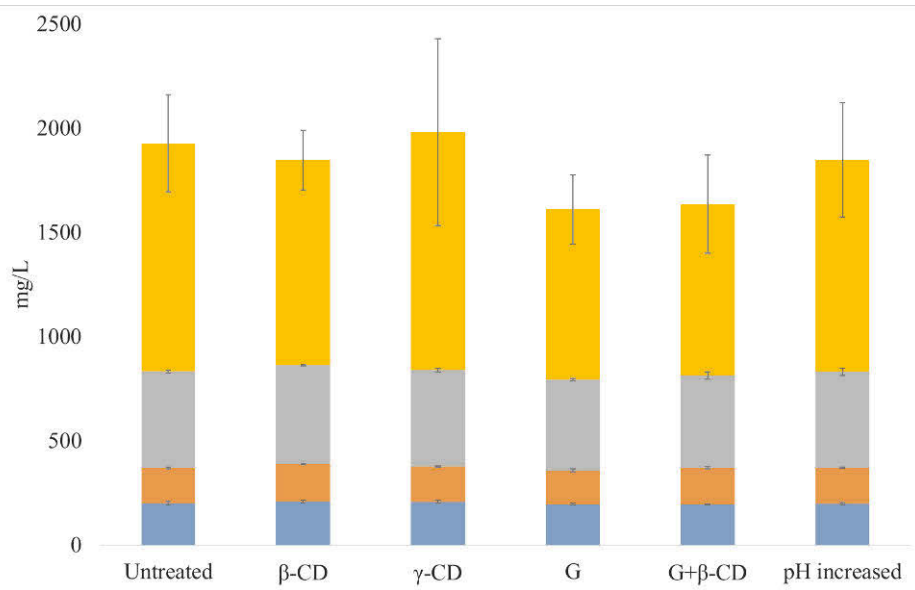

Figure 6. Sum of phenolic compound contents in the lingonberry juices (**I**). ■ Total anthocyanins, ■ total phenolic acids, ■ total flavonols, ■ total procyanidins with DMAC, *β*-CD *β*-cyclodextrin, *γ*-CD *γ*-cyclodextrin, G gelatine, G+*β*-CD sequential gelatine and β-cyclodextrin.

A principal component analysis (PCA) model was constructed with 26 chemical variables and 6 lingonberry juice samples. The PCA model with the first three principal components explained 87% of the total variation in the data (**Figure 7**). The gelatine treatment (G) negatively correlates with the chemical variables on PC1, whereas the *β*-CD sample correlates positively with the phenolic compounds, especially flavonols, compared to every other treatment. In addition, *β*-CD, *γ*-CD, and NaOH (pH increased) treated and untreated lingonberry juices negatively correlate with G and G+*β*-CD treated juices on PC1 indicating clear difference in the chemical composition. *β*-CD, *γ*-CD, and G treated and untreated juices positively correlate with each other and negatively with NaOH and G+*β*-CD treated juices on PC2. PC3 collects *γ*-CD and NaOH treated and untreated lingonberry juices together and separates *β*-CD from them. In addition, the majority of the chemical variables correlate positively more clearly with the *β*-CD on PC3. The difference between the CD treatments is clearly shown in the PCA figures. The difference may be caused by slightly different molar concertation of added CDs. However, the *γ*-CDs wider cavity is more feasible explanation, because it does not form as stabile an inclusion complex with small compounds, such as PAs, compared to *β*-CD (Szejtli, 1998b).

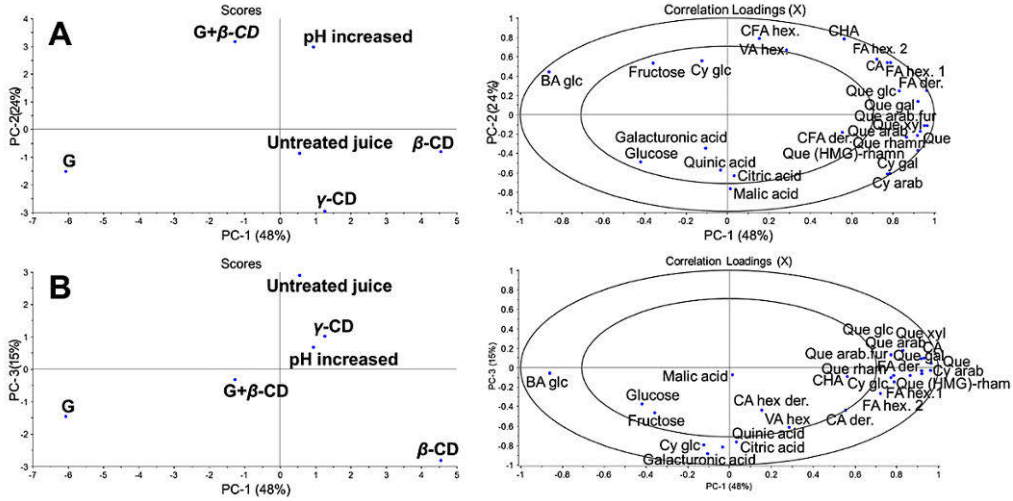

Figure 7. Principal component analysis model based on the chemical variables (n = 26) in the lingonberry juice samples (n = 6). **A**) PC1 and PC2; B) PC1 and PC3.

The sensory quality (*taste* and *astringenct mouthfeel*) of the polymer or NaOH treated lingonberry juices was rated on a scale from 0 to 10. All the juices were evaluated by comparing them to the untreated lingonberry juice. The data was collected as frequencies of participants who detected an increase in *sweetness* or a decrease in *sourness*, *bitterness* or *astringent mouthfeel* properties in treated juices compared to the untreated juice. All the juices were evaluated as notably *sour*, *bitter*, and *astringent* with very little *sweetness*. Interestingly, the juices with two treatments (*β*-CD with gelatine treatment or higher pH) were detected more often as being different in comparison to the juices with only one treatment (e.g. gelatine treated or CD) indicating treatments with CDs alone may not be sufficient to affect the negative sensory attributes. The CD concentration used in this study was selected to be above the levels used by Konno et al. (1982), and Gaudette and Pickering (2012a, 2012b). However, the efficacy of the bitter blockers is highly dependent on the bitter compound (Gaudette and Pickering, 2012a). *β*-CD has been reported to successfully mask the bitter taste in concentrates of grape fruit and mandarin juices in as low as 3 g/L concentrations (Szente and Szejtli, 2004). Lingonberries have a high content of benzoic acid (11 mM; (Visti et al., 2003)), the tate properties of which taste have been reported to be pungent and sour at the concentration of 6 mM in an aqueous solution (Otero-Losada, 1999). In addition, it has also been reported to have a prickling effect in the oral cavity (Otero-Losada, 2003). Due to the small size of benzoic acid and its hydrophilic properties, *β*-CD does not form stabile inclusion complexes with it (Szejtli, 1998b) indicating that *α*-CD could be more suitable for the modification of the taste properties of lingonberries.

5.3.2 Effects of enzymatic treatments on lingonberry juice (II)

All used enzymatic treatments significantly increased the lingonberry juice yield. With the three-way ANOVA, the enzyme type was more highly significant for juice yield than were the effects of incubation time or dosage. The highest juice yield was obtained using RPC (80.2–81.2%) with mainly pectinolytic activity, and the lowest yields resulted from RMC (68.7–74.7%) with mainly cellulolytic activity or NF10 (68.9–72.4%) with mainly pectinolytic activity. Similar differences in the juice yields have been reported for blueberries (Siddiq et al., 2018) and for bilberries and black currants (Buchert et al., 2005) by using pectinases and cellulases. Surprisingly, a three-hour heat treatment without enzyme addition (WO3h) produced a significantly greater juice yield than the one-hour heat treatment without enzyme addition (WO1h) (by 75.2% and 63.3%, respectively). In addition, a one-hour heat treatment resulted in a lower juice yield compared to the juice without any treatment. Incubation time and dosage had less notable but significant impacts on the yields, with higher dosage or a longer incubation generally resulting in higher juice yields. However, in both cases, the interaction effects with the enzyme type were statistically significant, thus the impact of dosage and time were dependent on the selected enzyme. A PCA model was constructed using 23 enzymatic treated samples and 41 non-volatile chemical variables (Figure 8). The juice yield negatively correlates with most of the samples on PC1, because it has a strong positive correlation with one-hour RPC enzyme product treatments.

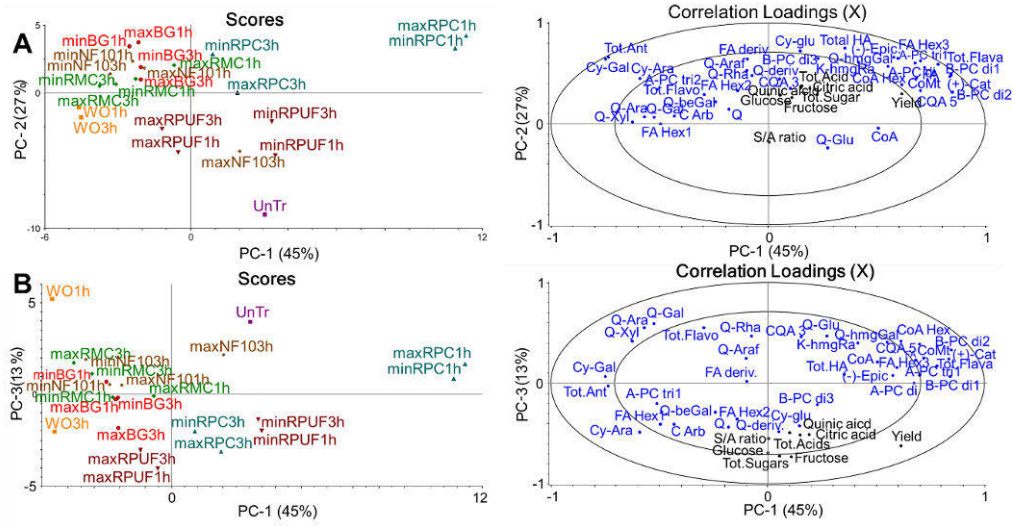

Figure 8. Principal component analysis of 23 treated samples (**II**) **A**) PC1 vs PC2, **B**) PC1 vs PC3; ■ untreated lingonberry juice, ■ without enzyme, ● β-glucosidase, ♦ cellulase, ▲ pectinase, ▼ pectinase, ❋ pectinase; 45 nonvolatile chemical variables (blue = phenolic compounds, black = sugars, organic acids, and juice yield). min/max = minimum or maximum enzyme dosage, 1 h/3 h = one-hour or three-hour incubation time.

Anthocyanins and flavan-3-ols were the main flavonoid classes, representing 45 and 32% of total flavonoids, respectively, in the non-treated lingonberry juice. Figure 9 represents the total amounts of phenolic compounds in all lingonberry samples. After maxRPUF3h treatment, the lingonberry juice exhibited the lowest amount of all total phenolic compounds, whereas maxBG1h treatment resulted in the juice with the highest total anthocyanin, maxRPC1h treatment had the highest total flavan-3-ols and phenolic acid contents, and maxNF103h treatment the highest total flavonol content. However, all treatments significantly increased the total anthocyanin content compared to untreated juice, but this phenomenon was not observed with any other phenolic compound group.

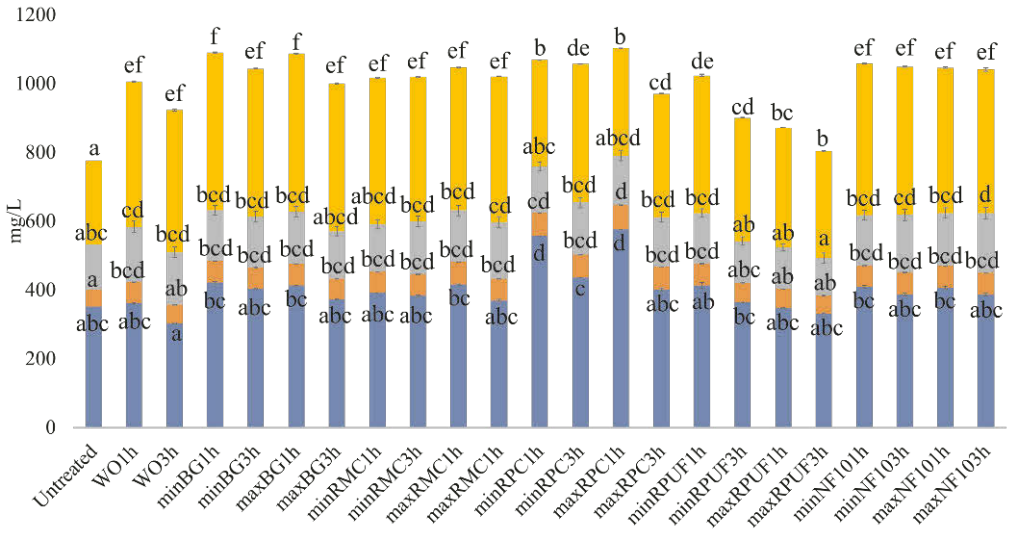

Figure 9. Total non-volatile content in the untreated, heated, and enzymatic treated lingonberry juices. ■ total flavan-3-ols, ■ total phenolic acids, ■ total flavonols, ■ total anthocyanins. min/max = minimum or maximum enzyme dosage 1 h/3 h = one-hour or three-hour incubation time.

The enzyme type main effect was again more significant for the anthocyanin contents than the dosage and time main effects in the three-way ANOVA model. Moreover, the addition of enzymes significantly affected the content of total anthocyanins compared to the untreated juice. The most notable effects on the anthocyanin content were observed with BG, NF10, and RMC enzymes (increases of 188%, 180%, and 174%, respectively) compared to the untreated lingonberry juice. The PCA model (**Figure 8**) shows the positive correlation of the total anthocyanin content, cyanidin galactoside, and cyanidin arabinoside with these enzyme products. Interestingly, only some treatments with BG or NF10 increased the anthocyanin content compared to the juice with thermal incubation but no enzyme addition. In addition, some pectinase (RPC and RPUF) enzyme-aided juices in this study showed a

lower content of anthocyanins than the WO juices. This result was consistent with the study by Viljanen et al. (2014), where they reported a 55% decrease in anthocyanin content when using a pectinase to prepare lingonberry juice. The greatest decreasing effect of the enzymatic treatments was observed with the principal anthocyanin, cyaniding galactoside, which may be the result of the high galactosidase side activity of the enzymes (Buchert et al., 2005; Viljanen et al., 2014). However, further statistical analysis with an independent sample t-test showed that the longer incubation time of RPC significantly increased the content of cyanidin galactoside, cyanidin arabinoside, and the total anthocyanins and reduced the cyanidin glucoside content. A longer incubation time with RPUF significantly elevated the content of cyanidin glucoside and cyanidin arabinoside and decreased cyanidin galactoside and the total anthocyanin content. It is worth noting that the impact of incubation time on the anthocyanin content was stronger with RPC than with the other enzymes. Finally, the cyanidin arabinoside level decreased more with longer a incubation time. Previously studies have reported anthocyanin pentosides to be more sensitive to processing than anthocyanin hexosides (Trošt et al., 2008; Wilkes et al., 2014).

In the volatile profiles, the same compounds were detected in all the treatments; however, individual contents exhibited some variations as shown in the PCA models. The RPUF samples were excluded from the PCA due to their domination on all the volatile variables (except for octanal). A higher content of volatiles with RPUF are linked to the higher pectinase activity, as described previously, leading to a major release of bound volatiles. After excluding the RPUF samples, the first two components explained 85% of variance (**Figure 10**). Juices prepared with 3-hour incubation of NF10 or BG enzymes are separated from the 1-hour incubated samples, suggesting that these enzymes were more dependent on time rather than on enzyme dosage. Similar separation is not observed in the RPC or RMC samples, indicating that little variation was caused in the volatile composition by these enzymes. The non-enzyme treated samples correlate negatively with the volatiles on PC1, suggesting that most of the studied volatiles are increased by using enzymatic assistance in juice processing. However, half of the enzymatic treatments also correlate negatively with the volatile compounds indicating a higher increase of the volatiles with some enzymatic treatments, such as the three-hour incubation with NF10 or BG. PC2 shows some separation of BG and RPC with a higher content of methyl benzoate from RMC, NF10 and the WO samples. In their research, Viljanen et al. (2014) compared the impact on the volatile profile of enzymatic treatment by employing a mixture of pectinase and protease with the effect of fermentation using lactic acid bacteria (LAB) and yeasts. They concluded that there was only a moderate elevation of the volatile contents with enzymatic treatments, whereas major changes were produced by yeast fermentation.

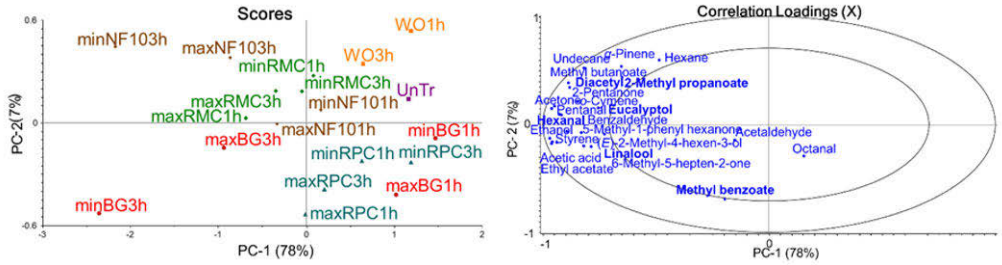

Figure 10. Principal component analysis of 23 treated samples; ■ without enzyme (WO), ● β-glucosidase (BG), ♦ cellulase (RMC), ▲ pectinase (RPC), ▼ pectinase (RPUF), ❋ pectinase (NF10), 24 volatile chemical variables (blue), **bold** volatiles detected with GC-O. min/max = minimum or maximum enzyme dosage, 1 h/3 h = one-hour or three-hour incubation time.

5.3.3 Effects of wine yeast fermentations on the black currant juice (III and IV)

The highest ethanol content was observed in SC2 (4.47 %, v/v) and the lowest in MPSC2 (3.46 %, v/v). The ethanol concentration in the SC2 fermented beverage was statistically significantly higher than after all sequential fermentations (3.46–3.79 %). Typically, *T. delbrueckii* is used to reduce the ethanol content in grape wines (Contreras et al., 2015), whereas our study demonstrated that a higher ethanol content (4.07 %, v/v) was found after the TD fermentation than in the SC1 fermented sample (3.84 %, v/v).

An independent sample t-test was used to compare the non-volatile and volatile composition of the black currant juice and the averaged value of all fermented black currant beverages. Generally, both profiles differed after fermentation compared to the black currant juice.

Seven organic acids and three sugars were detected in the juice and fermented beverages. The fermentations primarily decreased the concentrations of malic, shikimic, and quinic acids. *Saccharomyces* yeasts have reported to have malic acid degradation properties during fermentation (Redzepovic et al., 2003). The citric acid content was maintained at the same level. Succinic acid and galacturonic acid were not present in the black currant juice, but they appeared in the all the fermentations. The SB sample had the highest succinic acid level (0.9 g/L), and the sequential fermentations had the highest galacturonic acid levels (0.3 g/L). Yeasts produce succinic acid during alcohol fermentation through a Krebs cycle (Waterhouse et al., 2016) and galacturonic acid is formed as a degradation product of pectin by pectinases. Both of these organic acids may effect the taste properties: taste of succinic acid has been reported to be sour, bitter, and savory and galacturonic acid to have a sour taste (Da Conceicao Neta et al., 2007). The detection threshold for the sour taste of succinic acid is 0.7 mmol/L and for savory 0.9 mmol (Methven, 2012), and the detection threshold for the sour taste of galacturonic acid has been determined to be 0.643

mmol/L (Hufnagel and Hofmann, 2008). Both, succinic and galacturonic acid, had higher levels than the detection thresholds, 4.2–7.6 and 1.0–1.5 mmol/L, respectively, which probably had an effect on the final taste properties.

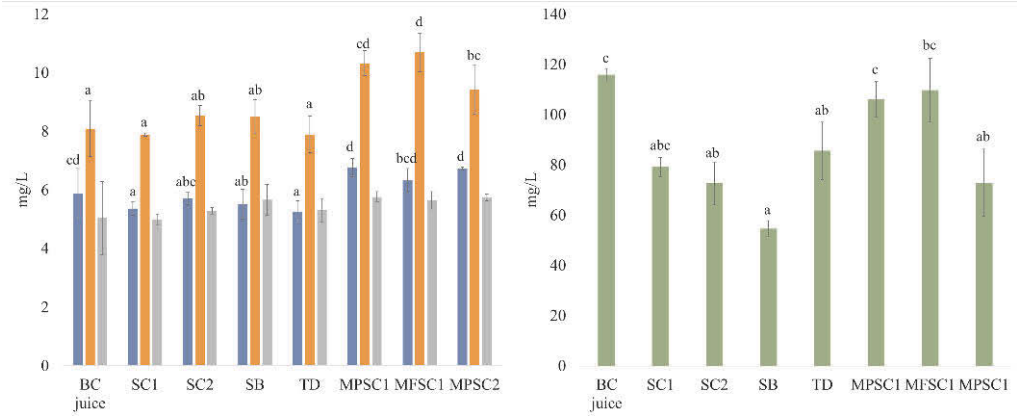

Figure 11. The total contents of the phenolic compounds in the black currant juice and fermented beverages. ■ total phenolic acids, ■ total flavonols, ■ total flavan-3-ols, and ■ total anthocyanins.

Fermentation decreased the total concentration of hydroxycinnamic acids (by 6.7 %) and the total content of anthocyanins (by 28.4 %) and increased the total concentration of flavonols (by 11.7 %) and flavan-3-ols (by 8.8 %; **Figure 11**). The loss of monomeric anthocyanins can be attributed to variations in either anthocyanin absorption by the yeast cell wall or *β*-glucosidase activity, which are both yeast characteristics, depending on the yeast species and strain (Blazquez Rojas et al., 2012; Hong et al., 2019; Morata et al., 2019b). *β*-Glucosidase activity can also effect on the content of other flavonoids with glycoside unit. The contents of phenolic acids with the nitrile group decreased 1.5 to 19-fold during the fermentations, and one of the nitrile compounds was detected only in trace amounts. Two new nitrile compounds (**Table 8**, compounds 25 and 26) were detected in the fermented black currant beverages and their content significantly differed between fermentations: the sequentially fermented beverages had a significantly higher content of both compounds than the single yeast fermented beverages. This may be the result of the high *β*-glucosidase activity of *Metschnikowia* yeasts (Morata et al., 2019b). In addition, the concentrations of the initial compounds (**Table 8**, compounds 22 and 23) decreased 1.5 to 15-fold. The highest decrease was observed with SC1 by 93.5 % and 65 %, respectively. However, the content of *Z*-*p*-coumaroylglucose, ferulic acid hexoside, and *p*-coumaric acid were significantly higher in the fermented beverages than in the juice, which is in contrast to the result reported by Czyzowska & Pogorzelski (2002). They observed a decrease of phenolic acids by half after the fermentation of black currant juice.

The colour properties, intensity, and tonality of the fermented beverages were analysed with a spectrophotometer. The highest colour intensity was recorded in the black currant juice. Of the fermented beverages, the SC2 had the highest colour intensity, and the SB had the lowest. The sequential fermentations produced more intense colour compared to the SC1, SB, and TD beverages. A decreased blue colour proportion and a increased proportion of yellow and red colours were detected in the beverages with lower colour intensity and lower total anthocyanin content. In general, all the monomeric anthocyanin content decreased during the fermentations (**Figure 11**): in the black currant juice, the total anthocyanin content was 1159 mg/L, and after the fermentations, the total anthocyanin content varied between 637 (SB) and 1153 mg/L (MPSC1), corresponding to 45 and 0.5 % loss in the content. The total anthocyanin contents of the sequential fermentations did not differ statistically from the non-fermented black currant juice or from each other, whereas all the fermentations with single yeast strains did. Czyżowska and Pogorzelski (2004) reported a loss of 96 % of the anthocyanins after the pectinase treated black currant juice was fermented with the wine yeast, which is a notable higher loss than in the Study **III**. Belda et al. (2016) and Escribano-Viana et al. (2019) have reported a higher anthocyanin content after sequential fermentation with *M. pulcherrima* and *S. cerevisiae* compared to the pure *S. cerevisiae* fermentation. They observed differences in the colour properties and the loss of monomeric anthocyanin during the fermentations. The differences can be attributed to variations in either *β*-glucosidase activity or anthocyanin absorption by the yeast cell wall among the different yeast strains.

To visualise the relationships between the black currant juice and fermented beverages and between the single yeast fermented and sequentially fermented, and between *Saccharomyces* and non-*Saccharomyces* fermented beverages, two PCAs were constructed with one juice sample, seven fermented samples, and 63 or 62 non-volatile chemical variables (**Figure 12A** and **B**, respectively). First PCA (**Figure 12A**) shows that black currant juice is clearly separated from the fermented samples on PC1. Not surprisingly, the ethanol and total sugar contents correlate negatively with one another. The nitrile containing hydroxycinnamic acids (variables P20-P23), anthocyanins, and all three sugars correlate positively with black currant juice (BJC). The aglycones of the nitrile containing hydroxycinnamic acids (P24 and P25), ethanol (EtOH), and myricetin rutinoside (F1) correlate negatively with the black currant juice. In the second PCA (**Figure 12B**), PC1 clearly separates the single yeast fermentations from the sequential fermentations on the two sides of the plot. Between the sequential fermentations, there are still clear differences: MPSC1 and MFSC1 are located closer to each other than to MPSC2 and the separation is in both components. The correlation loadings plot shows almost all the chemical variables, especially flavonols and anthocyanins, are located on the left-hand side correlating with the MPSC1 and MFSC1 samples. In addition, galacturonic acid (H7) correlates positively with these

beverages and clusters most of the anthocyanins and the total amount anthocyanins. This classification can indicate higher pectinase enzyme activity and thus has positive effects on the anthocyanin content after sequential fermentations. SC1, SC2, TD, and SB beverages are primarily on the right-hand side of the plot. However, the SC1 and SB beverages are clearly separated on PC2, whereas the SC2 and TD beverages are in the middle area of the plot. In addition, certain of the chemical variables, such as *p*-coumaric acid-4-*O*-glucoside (P2), succinic acid (H1), and B-type procyanidin dimer (FL5), correlate positively with the SB. In addition, ethanol correlates the most with the SB sample. On the PCA (**Figure 12B**), the SC2 and TD beverages are located closer to each other than any other sample, and thus their sensory properties may be more similar to each other than the other samples. The SC1 sample correlates positively with fructose (S1), glucose (S2), total sugars, sugar and acid ratio (S/A), and anthocyanin aglycones (A10 and A12) indicating a more sweet beverage compared to other fermentations.

The third component separates the MPSC2 samples more from the other sequential fermentations and locates the SC beverages closer to each other and groups SB and TD samples closer to each other. This may indicate more similarity between SC fermented beverages compared to the other two single yeast fermentations. Most interestingly, MPSC2 clearly correlates negatively with the anthocyanins, the initial nitrile compounds (21-24), glucose, fructose, and succinic acid on PC3.

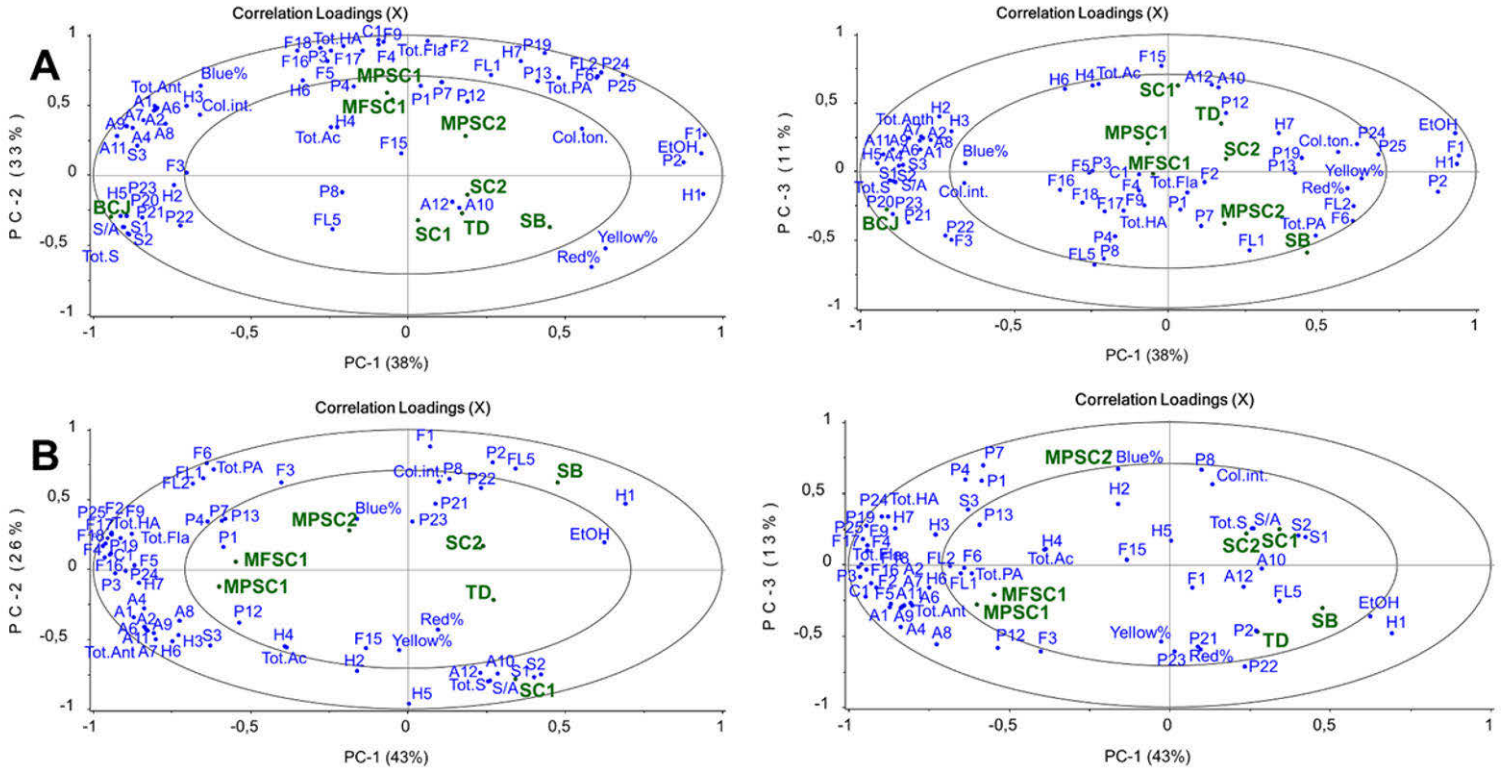

Figure 12. Principal component analysis based on 57 non-volatile chemical variables in the black currant juice and fermented black currant beverages using the following inoculation schemes: **A**) All samples, **B**) only fermented beverages. 1A succinic acid, 2A malic acid, 3A shikimic acid, 4A citric acid, 5A quinic acid, 6A ascorbic acid, 7A galacturonic acid, 1S fructose, 2S glucose, 3S sucrose, the numbers of the variables refer to Table 8.

Seventy-six volatile compounds were quantified from the fermented beverages (**IV**), of which 47 differed statistically significantly between the yeast strains used. The total content of the volatile compound groups are shown in the Figure 13. The most abundant volatile group were the higher alcohols contributing 66.1–74.6 % of the total volatile compounds. The second most abundant group was the fatty acids (10.6–16.0 %) and the third was the acetate esters (6.3–9.4 %).

The wide range of the total higher alcohols were determined in the FBs: the SB fermentation resulted in the highest (4154 µg/L) and the SC1 fermentation the lowest content (2667 µg/L). The sequential fermentation with the MPSC1 resulted in a similar total higher alcohol level than SC1, whereas the other sequential fermentations resulted in notably higher levels compared to their corresponding single yeast fermentations.

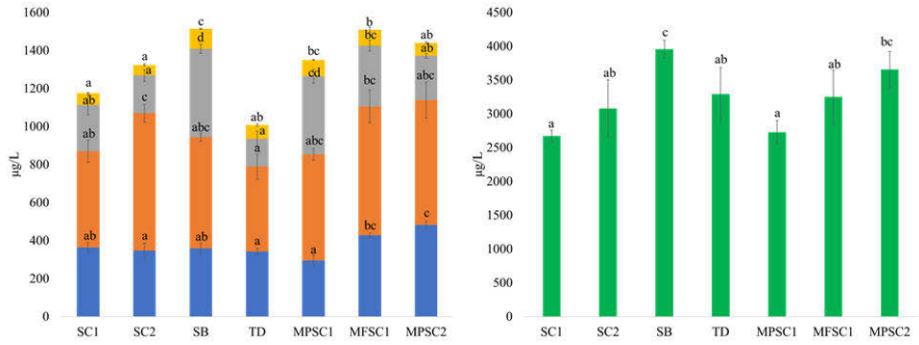

Figure 13. Total contents of volatile compound groups in the fermented black currant beverages. ■ total acetates, ■ total fatty acids, ■ total esters, ■ total terpenes, and ■ total higher alcohols.

The total acetate contents varied between 294 µg/L (MPSC1) and 483 µg/L (MFSC2). Ethyl acetate was the most abundant acetate in all samples. It represented 42–87.8 % of the total acetate contents. The highest concentration of ethyl acetate was observed in the MPSC2 (336 µg/L, 69.5 %) and the lowest in the SB (151 µg/L, 42.2 %). This result is consistent with the study of Canonico et al. (2019), where they observed sequential fermentation of *M. pulcherrima* and *S. cerevisiae* to produce notable higher ethyl acetate content compared to a single yeast fermentation with *S. cerevisiae*. *S. cerevisiae* yeasts have been reported to produce at least a 1.4-fold higher amount of ethyl acetate than *S. bayanus* when fermenting raspberry juice (Duarte et al., 2010). Here, *S. cerevisiae* fermentations resulted in 0.7 times higher ethyl acetate contents than *S. bayanus*. The excess concentration of ethyl acetate may have a negative impact on the sensory properties of the wine (Lambrechts and Pretorius, 2000). Ethyl esters were the main esters in the FBs contributing 79–96 % of all esters.

The total ester content varied between 148 µg/L (TD) and 466 µg/L (SB). Surprisingly, TD had the lowest ester production performance of all the yeasts despite previous studies having reported its high ester production. However, Liu et al. (2019) studied two *T. delbrueckii* strains, of which one had a similar ester production performance as in our study. The sequential fermentations with MP resulted in a statistically significant higher ester content than the corresponding single yeast fermentations with SCs, whereas the sequential fermentation with MF resulted in a slightly lower level. Ethyl octanoate (33–123 µg/L) was the most abundant ester in all FBs.

A wide range of fatty acids were observed in the fermented beverages: the lowest level was in the TD (446.3 µg/L) and the highest in the SC2 (721.9 µg/L). All detected fatty acids belonged to the mid-chain fatty acids (4–12 carbons). Both straight and branched fatty acids were detected subsequent to all the fermentations (**Table 10**). Straight fatty acids are by-products of fatty acid metabolism (Waterhouse et al., 2016) and branched fatty acids are produced from branched amino acids via the Ehrlich pathway (Styger et al., 2011). The highest concentration of branched fatty acid was observed in the SC2 (30.6 µg/L) and lowest in SC1 (2.2 µg/L). The MPSC2 beverage had the highest amount of straight fatty acid (250.4 µg/L) and the TD beverage had the lowest (94.8 µg/L). Comitini et al. (2011) reported significantly lower volatile acidity when *M. pulcherrima* was used in the simultaneous fermentation with *S. cerevisiae*, which is not consistent with our results. However, many environmental properties, such as the level of unsaturated fatty acids, temperature, and amount of raw material solids in the juice, have an effect on the formation rate of the mid-chain fatty acids (Waterhouse et al., 2016). In all FBs, the fatty acid contents were so low they may not had any impact on the flavour properties.

To visualise the relationships between the black currant juice and the fermented beverages and between the single yeast fermented and sequentially fermented, and between *Saccharomyces* and non-*Saccharomyces* fermented beverages, two PCAs were constructed with one juice sample, seven fermented samples, and 82 or 66 volatile variables (**Figure 14A** and **B**, respectively). The chemical variables not detected after fermentations were excluded from the second PCA. First three PCs of the first PCA explained 83 % of the data variance (**Figure 14A**). The juice sample is separated and correlates negatively with the fermented beverages on PC1. On PC2, the SB correlates negatively with the other FBs. The Correlation Loadings plot shows how certain volatile compounds correlate with the juice while others correlate with the fermented beverages. PC3 hardly effects the juice sample, but it separates the TD sample from the other samples and locates the SB among the other beverage samples.

In the second PCA (**Figure 14B**), first three PCs explain 75 % of the data variance. PC1 separates SB from the other fermented beverages. PC2 clearly groups other fermentations into the sequential fermentations, *S. cerevisiae* fermentations, and *T. delbrueckii*, indicating the similarity inside the groups and the differences between the groups. A few volatile variables, such as acetic acid (variable V9), ethyl propanoate (V52), and *E*-linaloloxide (V83), exhibite positive correlation with SC beverages on PC1 and 2. On PC3, only acetic acid, total amount of fatty acids (Tot.FA), and 3-methylbutanal (V37) clearly correlate with SC beverages. Nineteen volatile variables, such as 2,3-butanediol (V24), 1,8-cineol (V81), heptanol (V28), and 3-methylbutyl pentadecanoate (V68), can be seen clustered near the SB, and the PC3 has only a slight influence on them. PC3 separates the SCs further from hte TD and a clear species differences can be seen. The PCA clearly shows the importance of *M. pulcherrima* (MP) for the sequential fermentations: MPSc1 and MPSC2 are located closer to each other than to the MFSC1 on PC2. Surprisingly, PC3 separates MPSC1 and MPSC2 from each other, thus locating MPSC2 close to the SC samples and MFSC1 and MPSC1 close to the TD sample. On PC3, straight fatty acids, such as hexanoic (V14), octanoic (V15), and decanoic acid (V16), and ethyl acetate (V2) correlate positively with MPSC2. All these compounds may have a negative impact on the sensory properties in high concentrations. However, methyl butanoate (V53), ethyl butanoate (V56), ethyl hexanoate (V52), pentanol (V22), nonanal (V47), octanal (V45), and *E*-linalooloxide (V83) relocates on PC3 to correlate with the MPSC1 and MFSC2. These compounds may have a positive effect on the FBs sensory properties.

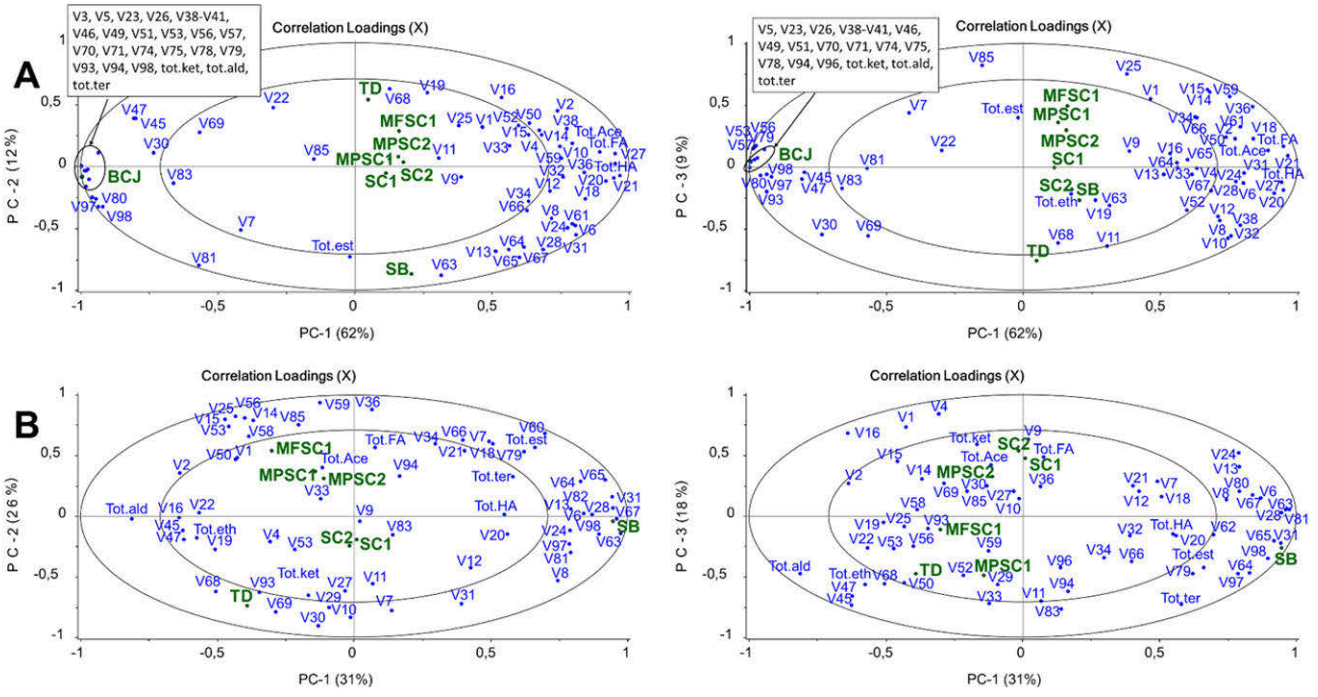

Figure 14. A) Principal component analysis of average values of biological replicates (n=8) of black currant juice and the fermented beverages as dummy variables (green) and chemical variables (n=84) with three components. **B**) Principal component analysis of average values of biological replicates (n=7) of the fermented beverages as dummy variables (green) and chemical variables (n=66) with three components. Numbers of the variables refers to the Table 10.

The BCJ, SB, and TD beverages were chosen for the GC-O analysis based on the differences observed in the volatile analysis. A total of 51 compounds contributing to the aroma were detected and described by the GC-O by two or more panellists (NIF ≥ 40 %; **Table 11**). Thirty-five of the detected scents were able to be paired with the compound identified by using retention indices and the MS data. The odour descriptions and external standard compounds were used to validate the GC-O identification.

Table 11. The volatile compounds identified with GC-O in black currant juice and fermented beverages.

		BCJ		SB		TD		
Compound*	**RI[a]**	**NIF %**	**SNIF**	**NIF %**	**SNIF**	**NIF %**	**SNIF**	**Description[b]**
n.i	542	50	106					Sweat
n.i	556					50	122	Alcohol, fresh, sweet
2,3-Butanedione	597			40	16			Butter, sweet, soft toffee
2-Methyl butanal	661	40	8	40	16			Musty
n.i	669	60	170					Roasted, stuffy, urine
Propyl acetate*	704	40	32					Green, grass, fresh
n.i	729	50	114					Sweat, stinky, musty
3-Methyl/2-methyl butanol	745			60	**354**	70	**332**	Musty, pungent, rancid
Pentanol	766			40	64	60	220	Sweet, fruity, candy
n.i	786	40	96					Plastic, pungent, sweet
Hexanal	805	50	112					Grass, green, leaf
Ethyl butanoate	808	50	116	70	200	50	**312**	Sweet, candy, fruity
n.i	824	70	194					Stuffy, roasted, mushroom
3-Methylbutanoic acid	831			60	192	40	80	Solvent, pungent, chemical
Methyl 3-hydroxy-butanoate[d]	857	50	178	60	124	50	188	Sweet, fruity, floral
3-Hexe-1-ol	867	40	64					Sweet, fruity, rhubarb
2-Furamethanol[d]	869					50	280	Popcorn, roasted, baked
1-Hexanol	872	60	164	80	**262**	70	**364**	Urine, musty, baking, cheese
3-Methylbutyl acetate	882			50	114	50	58	Fruity, pear, sweet
Heptanal	906	60	**382**					Leaf, green, flower
n.i	908	50	204					Mushroom, mould, earth
Ethyl 2-hydroxy-butanoate[d]	909			50	68			Sweet, floral, green
Methional	917	50	264	40	16	40	16	Potato, cheese, musty
n.i	925	60	**414**	60	238	50	**286**	Sweat, pungent, musty
Heptanol	970	50	130	50	26	60	188	Musty, pungent, spoiled
1-Octen-3-one/1-octen-3-ol	985	70	**400**	60	202	50	**294**	Mushroom, earthy, pungent
β-Myrcene	990	70	**518**	40	80	60	246	Raw carrot, metallic, chemical
Ethyl hexanoate	1005			60	**304**	50	182	Sweet, fruity, pineapple
Octanal	1006	70	**362**					Citrus fruit, green, lemongrass
1,8-Cineol	1034	40	16	40	8			Eucalyptus, mint, pastille
β-Ocimene	1051	40	64					Chemical, pungent, sweet
E-2-Octenal	1063	50	162					Lemongrass, green, grass
p-Cymenene[d]	1102	60	162	50	82	40	72	Pungent, solvent, green
Linalool	1106	50	220	60	178	50	222	Fresh, leaf, green
Rose oxide	1108	60	120	40	8	50	92	Fresh, rose, floral
Phenylethyl alcohol	1126			50	42	40	8	Rose, floral, perfume
E-2-Nonenal	1161	60	326					Cucumber, green, plant
n.i	1168	50	108					Leathery, floral, fresh
n.i	1171	50	208					Green, grass, soap
n.i	1182	60	72			40	48	Musty, roasted, bread
p-Cymen-8-ol[c]	1189	40	48			50	76	Herbal, grass, earth

		BCJ		SB		TD		
Compound*	**RI[a]**	**NIF %**	**SNIF**	**NIF %**	**SNIF**	**NIF %**	**SNIF**	**Description[b]**
Terpinen-4-ol[d]	1194	60	252	50	102	50	156	Herbal, pungent, bell pepper
Terpineol[d]	1201	50	130					Liquorice, anise
Ethyl octanoate	1213			40	**344**	40	160	Sweet, passion fruit, candy
Bornyl acetate	1265	40	112					Liquorice, anise, herbal
n.i	1311	40	24					Plant-like, raw carrot, metallic
n.i	1345	40	152					Plant-like, herbal, dried grass
β-Damascenone	1398	40	8					Berry-like, rowanberry, honey
3-Methylbutyl octanoate[d]	1427			40	**322**	50	186	Fruit, berry-like, honey
n.i	1428	60	346					Sweet, berry-like, honey
n.i	1455	50	164					Vanilla, sweet

* Identification with external standards; [a] Linear retention indices; [b] Three most frequently given description by panellists (n=5); [c] identification by RI and descriptors in Marsol-Vall et al. (2019b); [d] identification based only on MS data; bold numbers are the five highest SNIF values in the sample. BCJ black currant juice, SB *S. bayanus* fermented, TD *T. delbrueckii* fermented.

The BCJ had the highest count of detected compounds (39; Table 11) in the GC-O analysis, of which 25 were paired with an identification. Twenty-eight compounds were detected in the FBs, of which 22 were detected in both fermented samples. Only 11 compounds were detected in all samples. The SNIF values exhibited great differences between the samples. Almost all the SNIF values were notable lower in the FBs compared to the juice sample, but in a few cases, the SNIF value was higher at least in one FB. For example, the SNIF value of ethyl butanoate was 1.7 times higher in the SB and 2.7 times higher in the TD compared to the BCJ.

Fourteen compounds were only detected in the FBs (**Table 11**), of which two were in the TD beverage, one being an unidentified compound with RI 556 and and the other a 2-furamethanol; two were also found in the SB beverage, 2,3-butanedione and ethyl 2-hydroxybutanoate.

Many esters and terpenes, such as methyl butanoate, ethyl butanoate, ethyl hexanoate, 1,8-cineol, *α*-pinene, and *β*-damascenone, have been reported to be important contributors to the black currant aroma (Iversen et al., 1998; Jung et al., 2017; Mikkelsen and Poll, 2002; Varming et al., 2004b). According to the SNIF values (**Table 11**), the five most potential aroma compounds contributing to the studied black currant juice were *β*-myrcene, an unidentified compound with RI 925, 1-octen-3-one, heptanal, and octanal. The most potential aroma contributing compounds in the SB fermented beverage were 3-methyl/2-methyl butanol, ethyl octanoate, 3-methylbutyl octanoate, ethyl hexanoate, and hexanol, and in the TD fermented beverage hexanol, 3-methyl/2-methyl butanol, ethyl butanoate, 1-octen-3-ol, and an unidentified compound with RI 925.

Descriptive analysis of five fermented beverages (SC1, SB, TD, MPSC1, and MFSC1) were performed with a trained panel (n=11; **IV**). Despite the clear difference in the content of the non-volatile (**III**) and volatile compounds (**IV**) between the fermented beverages, only three statistically significant differences were found in the sensory evaluations. All samples were described as intensively *sour*, which may explain small number of differences detected between them by panellists. Three-way ANOVA showed a significant sample effect only in the *black currant odour* ($p<0.05$)

and *musty odour* ($p<0.01$). In addition, further statistical analysis with one-way ANOVA with an LSD test showed statistically significant difference ($p<0.05$) in *viscous mouthfeel*. The statistical difference in the *black currant odour* was between the SB (intensity of 4.6) and TD (5.6) beverages, the *musty odour* between SB (intensity of 4.3) and all the other beverages (intensities between 2.1–2.6), and the *viscous mouthfeel* between SB/MFSC1 (intensities of 3.8 and 3.7, respectively) and the TD beverages (3.1).

The partial least square regression model (PLS; **Figure 15**) was constructed with the non-volatile (**III**) and volatile chemical variables (**IV**) and their total amounts (X, n=125), the sensory evaluation attributes (Y, n=12), and the fermented beverages as dummy variables (n=5) in order to detected compounds contributing to different sensory characteristics. The three first PLS factors explained 89 % (X) and 88 % (Y) of the variance of the data. Factor-1 separates the sequential fermentations from all the single yeast fermentations. On the other hand, facto-2 separates TD from all the other FB samples. As mentioned previously, all the samples were described as *intensively sour*. A PLS regression model showed the positive correlation between the *sour taste* and the *total flavour intensity*. In addition, many flavonols and phenolic acids, such as myricetin glucoside (F2), *p*-coumaroyl quinic acid (P7), and nitrile containing phenolic acid aglycones (P24 and P25), and sugars (S1–S3) correlate positively with the *total flavour intensity*. From the beverage samples, MPSC1 and MFSC1 have the most positive correlation with the *flavour intensity*, flavonols, and the total amount of anthocyanins (Tot.Ant), and thus also with the colour intensity. The *sweet odour* correlates positively with some volatile compounds, such as nonanal (V47) and octanal (V45). Octanal was also detected in the GC-O analysis in the black currant juice with fifth highest SNIF value (SNIF 362). In the PLS regression model, octanal is also located near to the *black currant odour* and *taste*, indicating its contribution to these attributes. Octanal and nonanal are not abundant compounds in black currant berries (Liu et al., 2018; Varming et al., 2004a), but Varming, et al. (2004) observed 5.7 and 6.4 times higher octanal and nonanal contents after heating black currant juice at 90 °C for 60 min. In our Study **IV**, the black currant juice used was pasteurised at 97 °C for 30 s. The heating of the juice to 97 °C, took several minutes, which could explain the possible considerable importance of octanal and nonanal to the sensory attributes.

A clear negative correlation can be observed between the *viscous mouthfeel* and the galacturonic acid content. Galacturonic acid (variable H3 in **Figure 15**) is formed during pectin degradation by pectinolytic enzymes. The pectinase activity of wine yeasts is dependent on the strain and species. Belda et al. (2016) observed that many *M. pulcherrima* and *M. fructicola* strains exhibit polygalacturonase activity and You et al. (2016) observed pectinase activity in some *T. delbrueckii* strains. This is in consistent with our Studies **III** and **IV**, where the galacturonic acid content is highest after sequential fermentations with *Metschnikowia* yeasts and *S. cerevisiae* and single

yeast fermentation with *T. delbrueckii*. These beverages also correlate negatively with the *viscous mouthfeel* in the PLS regression model (**Figure 15**). In addition, the *black currant odour* and *flavour*, and *sweet odour* and *taste* correlate positively with the galacturonic acid. All these attributes could be desired attributes in the fermented black currant beverage. Furthermore, galacturonic acid correlates negatively with a *bitter taste* and *astringent mouthfeel* on factor-1. These attributes are not desired in the berries (Laaksonen et al., 2016).

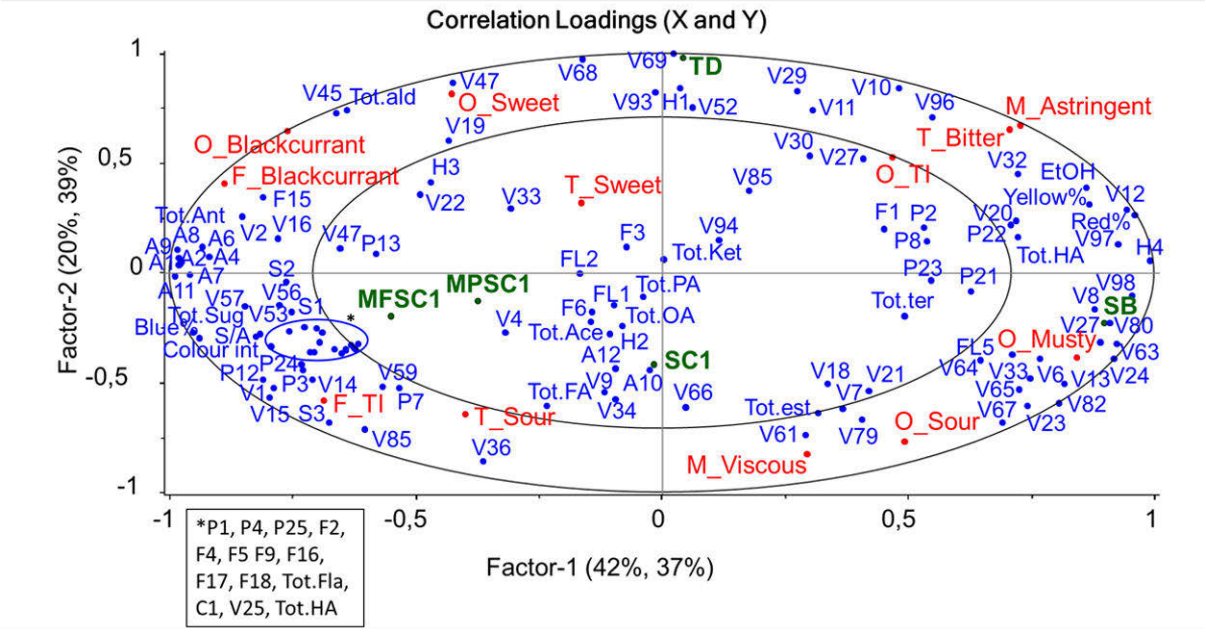

Figure 15. Partial least regression of the chemical variables and their total amounts (X, blue, n=125), sensory evaluation attributes (Y, red, n=12), and fermented beverages as dummy variables (green, n=5). Variable numbers are referring to the Table 8 (non-volatiles) and Table 10 (volatiles). H1 succinic acid, H2 citric acid, H3 galacturonic acid, S1 fructose, S2 glucose, S3 sucrose, F flavour, M mouthfeel, O odour, T taste, TI total intensity

The *musty* and *sour odours* correlates positively with the SB fermented beverage. In addition, the *total intensity of odour*, *bitter taste*, and *astringent mouthfeel* correlate positively with the SB beverage. In the PLS regression model, the *musty odour* correlates with many of the volatile compounds, such as heptanol (V28), 2-methylbutanoic acid (V13), which may contribute to the musty odour, but it also correlates with some esters and terpenes, such as V8, V98, V80. From these compounds, heptanol was also detected in the GC-O analysis (**Table 11**) of the SB fermented beverage and was also detected in the TD sample with a SNIF value of 188. However, the source of mustiness may also be those compounds that the study was not able to determine, the reason for this being that the detection thresholds of many musty compounds are only found in a few nano- or micrograms (Callejón et al., 2016). Callejón et al. (2016) reported 1-octen-3-ol being one of the most important compounds contributing to mustiness in wines. In this study, 1-octen-3-ol was not detected due to 3-(methylthio)-1-propanol eluted at the same time. The crytolarant nature of *S. bayanus* may explain the high *musty odour* of the SB fermented beverage. Muñoz-Bernal et al. (2016) reported high concentrations of certain higher alcohols when they fermented synthetic must at under 25 °C compared to 13 °C. Our fermentation temperature was 22 °C.

5.4 General discussion

Lingonberry is commonly used in Finland and it has the highest yield of all wild berries in Finnish forests, but only approx. 10 % of the annual yield is collected and utilised. In addition, there are only a few studies concerning lingonberries and an especially small number about the sensory properties and their association with the chemical composition of the berries. In this research work, two strategies, biopolymers and enzymatic treatments, were applied in order to modify the chemical composition and, thus the sensory properties of lingonberries. In the biopolymer study, the effects on the sensory properties were measured by an untrained consumer panel. The biopolymers were not powerful enough to modify sensory properties alone and more research on the chemical composition of lingonberries as well research with different biopolymers is needed. More knowledge about the chemical composition of lingonberries and compounds contributing to their sensory properties, could help to choose the biopolymers that would modify the sensory properties better. The enzymatic treatments statistically significantly influenced both the non-volatile and volatile chemical composition of the lingonberries, indicating possible effects on the sensory properties. However, although the sensory properties were not measured, the changes in the chemical composition indicated a more *bitter taste* and an *astringent mouthfeel* due to increased levels

of phenolic compounds. The study also showed differences in the enzymes used to help juice producers otpimise lingonberry juice production.

In the studies concerning lingonberries, two different lingonberry products, a commercial juice concentrate and a self-pressed juice from frozen lingonberries, were used. This caused some problems in the comparison between these two studies because of the effects processing is known to cause on the chemical composition of berries. Processing may degrade products causing a reduction in the concentrations, but it may also increase the levels of some compounds. However, in the processing methods, the parameters used in juice processing, such as the temperature during the concentration process, are not known and this made the evaluation of the processing effects on the chemical composition impossible.

Black currant is the second most cultivated berry crop in Finland. It has distinct taste properties and a delicious aroma. The chemical composition of black currants is well known, and the effects of the processing methods on chemical composition are well studied. However, there are only a few studies on fermented black currant beverages and none on beverages fermented with non-*Saccharomyces* yeasts. In this study, Finnish black currants were fermented with *Saccharomyces* and non-*Saccharomyces* yeasts and the changes in the chemical composition was investigated. In addition, a descriptive sensory evaluation was done with a sensory panel trained for this study. Even though a clear difference was observed in the chemical compositions of the fermented black currant beverages, only a few differences were observed in the sensory properties according to the trained panel. However, the results provided important information about the fermentation of black currants with non-*Saccharomyces* yeasts due to their pectinase activity. Black currants have a high pectin concentration making the enzymatic treatments crucial for juice production. *Metschnikowia* yeasts are known for their high enzymatic activities, such as pectinase and glucosidase, making them useful in pectin reduction and release of the bound volatile compounds.

The black currant berries used to produce fermented black currant beverages were commercial black currants and the variety was not known. Commercial black currants were used because they are also used in the industrial processing. The citric acid and pectin contents are high in black currants making them sour tasting and viscous, respectively. The sour taste is problematic for many consumers. Fermentation depleted the sugars almost completely, which emphasised the sourness, making it even more intense. High pectin concentration can affect the effectiveness of fermentation and make it uneven. To resolve these problems, future research will have to be conducted with black currant varieties that have low citric acid and pectin contents. In addition,

the possible quality improving effects of pre-treatment with pectinase or other enzymes cannot be omitted.

β-cyclodextrin and its effects on the sensory properties of food products is well researched and known. However, in this study, *β*-cyclodextrin did not successfully decrease the undesirable taste properties when added to the lingonberry juice. It is possible that the concentration used was not high enough to sufficiently bind the quantity of bitter and astringent compounds required. In addition, the compound properties, such as hydrophilicity and molecular size, were not suitable to form inclusion complexes with *β*-cyclodextrin. The benzoic acid content in lingonberries is high and it is known to affect the sourness and puckering and prickling mouthfeels. In addition, lingonberries have a high content of procyanidins contributing to the mouth-drying and puckering astringent characteristics. In this study, procyanidins with a degree of polymerisation of up to 9 was detected in the lingonberry concentrate. For these large molecules, it is impossible to enter to the cavity of the cyclodextrin, and thus the unwanted sensory properties are not masked. However, the sequential treatment with the gelatine and *β*-cyclodextrin was sufficient to reduce the bitterness and both the mouth-drying and puckering astringency. More research is needed on large-ring cyclodextrins, which could form inclusion complexes with hte larger compounds, or combine two or more cyclodextrins.

Saccharomyces yeasts are the most utilised oenological yeasts. In this work, three *Saccharomyces* yeasts, two *S. cerevisiae* and one *S. bayanus*, were used. The beverage fermented with *S. bayanus* had the mustiest odour, which may have been the result of the non-optimum fermentation parameters. In future research, the fermentation parameters should be optimised for every yeast to result in the best final quality of wines. The high citric acid content of black currant makes the juice intensively sour and lowers the pH to under 3, thus complicating the fermentation process. Future research on fermented black currant beverages should also include sequential or pure fermentation with yeasts capable of degrading citric acid, such as *Pichia fermentans*.

In this research for this thesis, a wide range of chromatographical methods were used to determine the non-volatile and volatile compositions of the lingonberry and black currant samples. However, only two sensory evaluation methods were used to determine the effects of fermentation on the sensory properties and possible association with the changes in the chemical composition. In addition, two studies included GC-O analyses of volatile compounds, but in Study **II,** it was only used to validate the identifications from the GC-MS analysis. Enzymatic treatments were done by changing the enzyme dosage and incubation time. Statistically significant changes were observed in the content of the non-volatile and volatile compounds between the

treatments, indicating possible effects on the sensory properties. Further research should include different incubation temperatures as processing parameter since temperature has been reported to influence the sensory properties of enzyme-treated juices. In addition, sensory evaluations with a trained sensory panel would give more significance to an estimation of the quality of the enzymatic treated lingonberry juice.

6 SUMMARY AND CONCLUSION

The results of the practical work of this research thesis showed that the selected bioprocessing methods could be used to modify the chemical composition and sensory properties of Finnish lingonberries and black currants. Additionally, the research provided new insight into the means by which modifications can be achvieved on the chemical composition of these Nordic berries.

Cyclodextrins, gelatine, and NaOH were all used to treat the commercial lingonberry juice. The gelatine treatment decreased the content of the phenolic compounds, whereas cyclodextrin, especially the *β*-cyclodextrin treatment tended to increase the content of the phenolic compounds, suggesting a protective effect by cyclodextrin on these compounds from degradation during the juice processing. Even though the *β*-cyclodextrin did not significantly decrease the bitterness and astringency of the commercial lingonberry juice, the study provided important information about the use of cyclodextrins when attempting to modify the sensory quality of juice in beverage production. The methodology used was based on the literature, and the results suggested the important impact of the raw material on the outcome of the treatment. In this thesis, the content of *β*-cyclodextrin used was higher than those applied in the literature material but nevetheless it was not sufficient to decrease the unwanted sensory properties. The gelatine treatment was used alone and also sequentially with *β*-cyclodextrin. The sequential treatment resulted in a juice that had the most pleasant sensory properties. However, the removal of phenolic compounds may compromise the health benefits of the juices since they are known to have various beneficial effects on human health.

Five enzyme products, belonging to three enzyme types, were used to produce lingonberry juice. The enzyme type had a significant effect on the content of the phenolic and volatile compounds. In contrast, the incubation time (1h or 3h) exhibited effects on fewer compounds. Surprisingly, the enzyme dosage had a significant effect with only one pectinase enzyme on the phenolic compounds. Regarding the volatile compound profiles, the HS-SPME-GC–MS analysis exhibited an enhanced content particularly in the pectinase-treated juices compared to all the other treatments. In addition, a longer incubation time with the research pectinase and *β*-glucosidase enzymes increased the volatile content of the juices, whereas the volatile compounds in the samples treated with pectinase (RPC) and cellulase were not affected by the incubation time.

Clear difference was observed between *Saccharomyces*, non-*Saccharomyces*, and sequentially fermented beverages regarding phenolic and volatile compounds. Beverage fermented with *S. bayanus* showed the most significant difference from the other fermented beverages in each of the aspects analysed.

Regarding the phenolic composition, the sequential fermentations exhibited more dependence on *S. cerevisiae*, whereas the volatile composition of sequential fermented beverages were depended on the *Metschnikowia* species than the *S. cerevisiae* strain. Even though a clear difference was observed in the chemical composition of the wine yeasts used, very little difference was found in the sensory evaluations. This is most likely due to the high citric acid content of the fermented beverages, which probably masked the other sensory attributes. The citric acid content could be decreased by fermentation with wine yeast, such as *Pichia fermentans*, which is capable of using citric acid as an energy source.

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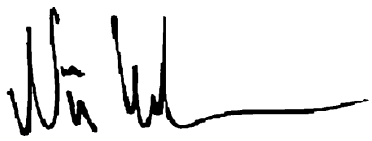

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APPENDIX: ORIGINAL PUBLICATIONS

I. Reprinted from the *LWT - Food Science and Technology* 2019, 113, 108295

II. Reprinted from the *Food Chemistry* 2021, 339, 128052

III. Reprinted form the *Journal of Agricultural and Food Chemistry* 2020, 68, 10128–10141

IV. Submitted

DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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

43. **TEEMU HALTTUNEN (2008)** Removal of cadmium, lead and arsenic from water by lactic acid bacteria.
44. **SUSANNA ROKKA (2008)** Bovine colostral antibodies and selected lactobacilli as means to control gastrointestinal infections.
45. **ANU LÄHTEENMÄKI-UUTELA (2009)** Foodstuffs and medicines as legal categories in the EU and China. Functional foods as a borderline case. (Law).
46. **TARJA SUOMALAINEN (2009)** Characterizing *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 as a new probiotic combination: basic properties of JS and pilot *in vivo* assessment of the combination.
47. **HEIDI LESKINEN (2010)** Positional distribution of fatty acids in plant triacylglycerols: contributing factors and chromatographic/mass spectrometric analysis.
48. **TERHI POHJANHEIMO (2010)** Sensory and non-sensory factors behind the liking and choice of healthy food products.
49. **RIIKKA JÄRVINEN (2010)** Cuticular and suberin polymers of edible plants – analysis by gas chromatographic-mass spectrometric and solid state spectroscopic methods.
50. **HENNA-MARIA LEHTONEN (2010)** Berry polyphenol absorption and the effect of northern berries on metabolism, ectopic fat accumulation, and associated diseases.
51. **PASI KANKAANPÄÄ (2010)** Interactions between polyunsaturated fatty acids and probiotics.
52. **PETRA LARMO (2011)** The health effects of sea buckthorn berries and oil.
53. **HENNA RÖYTIÖ (2011)** Identifying and characterizing new ingredients *in vitro* for prebiotic and synbiotic use.
54. **RITVA REPO-CARRASCO-VALENCIA (2011)** Andean indigenous food crops: nutritional value and bioactive compounds.
55. **OSKAR LAAKSONEN (2011)** Astringent food compounds and their interactions with taste properties.
56. **ŁUKASZ MARCIN GRZEŚKOWIAK (2012)** Gut microbiota in early infancy: effect of environment, diet and probiotics.
57. **PENGZHAN LIU (2012)** Composition of hawthorn (*Crataegus* spp.) fruits and leaves and emblic leafflower (*Phyllanthus emblica*) fruits.
58. **HEIKKI ARO (2012)** Fractionation of hen egg and oat lipids with supercritical fluids. Chemical and functional properties of fractions.
59. **SOILI ALANNE (2012)** An infant with food allergy and eczema in the family – the mental and economic burden of caring.
60. **MARKO TARVAINEN (2013)** Analysis of lipid oxidation during digestion by liquid chromatography-mass spectrometric and nuclear magnetic resonance spectroscopic techniques.
61. **JIE ZHENG (2013)** Sugars, acids and phenolic compounds in currants and sea buckthorn in relation to the effects of environmental factors.
62. **SARI MÄKINEN (2014)** Production, isolation and characterization of bioactive peptides with antihypertensive properties from potato and rapeseed proteins.
63. **MIKA KAIMAINEN (2014)** Stability of natural colorants of plant origin.
64. **LOTTA NYLUND (2015)** Early life intestinal microbiota in health and in atopic eczema.
65. **JAAKKO HIIDENHOVI (2015)** Isolation and characterization of ovomucin – a bioactive agent of egg white.
66. **HANNA-LEENA HIETARANTA-LUOMA (2016)** Promoting healthy lifestyles with personalized, *APOE* genotype based health information: The effects on psychological-, health behavioral and clinical factors.
67. **VELI HIETANIEMI (2016)** The *Fusarium* mycotoxins in Finnish cereal grains: How to control and manage the risk.
68. **MAARIA KORTESNIEMI (2016)** NMR metabolomics of foods – Investigating the influence of origin on sea buckthorn berries, *Brassica* oilseeds and honey.
69. **JUHANI AAKKO (2016)** New insights into human gut microbiota development in early infancy: influence of diet, environment and mother's microbiota.
70. **WEI YANG (2017)** Effects of genetic and environmental factors on proanthocyanidins in sea buckthorn (*Hippophaë rhamnoides*) and flavonol glycosides in leaves of currants (*Ribes* spp.).
71. **LEENAMAIJA MÄKILÄ (2017)** Effect of processing technologies on phenolic compounds in berry products.
72. **JUHA-MATTI PIHLAVA (2017)** Selected bioactive compounds in cereals and cereal products – their role and analysis by chromatographic methods.
73. **TOMMI KUMPULAINEN (2018)** The complexity of freshness and locality in a food consumption context
74. **XUEYING MA (2018)** Non-volatile bioactive and sensory compounds in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*)
75. **ANU NUORA (2018)** Postprandial lipid metabolism resulting from heated beef, homogenized milk and interesterified palm oil.
76. **HEIKKI AISALA (2019)** Sensory properties and underlying chemistry of Finnish edible wild mushrooms.
77. **YE TIAN (2019)** Phenolic compounds from Finnish berry species to enhance food safety.
78. **MAIJA PAAKKI (2020)** The importance of natural colors in food for the visual attractiveness of everyday lunch.
79. **SHUXUN LIU (2020**) Fermentation with non-*Saccharomyces* yeasts as a novel biotechnology for berry wine production.
80. **MARIKA KALPIO (2020)** Strategies for analyzing the regio- and stereospecific structures of individual triacylglycerols in natural fats and oils.
81. **JOHANNA JOKIOJA (2020)** Postprandial effects and metabolism of acylated anthocyanins originating from purple potatoes
82. **NIINA KELANNE (2021)** Novel bioprocessing for increasing consumption of Nordic berries.

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