Impact of cyclodextrin treatment on composition and sensory properties of lingonberry (*Vaccinium vitis-idaea*) juice

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Abstract

Lingonberry (*Vaccinium vitis-idaea* L.) juice was treated with 10 g/L cyclodextrins (CD) or gelatin as potential means to improve the stability of bioactive compounds and to modify the sensory properties. Before and after the treatment acids, sugars, and phenolic compounds (anthocyanins, flavonols, phenolic acids, proanthocyanidins) of the juices were analyzed with chromatographic and mass spectrometric methods. Forty-six phenolic compounds were identified or tentatively identified, of which twenty-four were A-type or B-type proanthocyanidins with degree of polymerization of 1 to 9. Juices treated with β-CD had the highest contents of phenolic compounds (phenolic acids 17.9 mg/100 mL, flavonols 47.5 mg/100 mL, anthocyanins 21.0 mg/100 mL, and proanthocyanidins 114.2 mg/mL), whereas gelatin treatment resulted in significantly lowered content of the compounds (phenolic avids 16.3 mg/100 mL, flavonols 43.50 mg/100 mL, anthocyanins 19.7 mg/100 mL, and proanthocyanidins 81.7 mg/mL). Taste and astringency attributes of the juices were investigated with untrained panelists (n=40). The lingonberry juices were perceived as notably sour, bitter and astringent. CD or gelatin treatments alone had less effect on the astringency of the juice in comparison to their use in combination. The results suggest that, CDs may be utilized as protectants of the phenolic compounds, especially anthocyanins, in lingonberry juices.

Keywords cyclodextrin, flavor modification, lingonberry juice, phenolic compound, sensory quality

1 Introduction

Fruits and berries are recommended to be part of daily human diet. Fresh and natural aroma are important quality factors of berry products (Deliza, Rosenthal, Abadio, Silva, & Castillo, 2005). Nevertheless, some berries, such as lingonberry (*Vaccinium vitis-idaea*), cranberry (*Vaccinium macrocarpon*), and sea buckthorn (*Hippophaë rhamnosides*), have intense flavors and are often too bitter and astringent for consumers to eat as such (Laaksonen, Knaapila, Niva, Deegan, & Sandell, 2016). The composition and contents of the phenolic compounds in fruits and berries may contribute to the unpleasant flavors, such as bitterness and astringency. The impact on sensory properties is often compound specific. For example, (–)-epicatechin is perceived as more bitter and astringent than (+)-catechin at the same concentration (Kallithraka, Bakker, & Clifford, 2007), whereas phenolic acids, such as *p*-coumaric acid and vanillic acid, are astringent, and short-chained procyanidins are both bitter and astringent (Hufnagel & Hofmann, 2008). Addition of sugar to berry products is an effective way to mask these unpleasant taste properties, but consumption of excess sugar decreases the health benefits of the berry products by causing, for example, weight gain and an increasing risk of type 2 diabetes (Schulze et al., 2004).

The lingonberry (*Vaccinium vitis-idaea*) is a common Nordic berry, which has potential health benefits due to anti-inflammatory, an anti-antherothrombotic, hypoglycemic profile, and antioxidantive activities (Kivimäki et al., 2012; Määttä-Riihinen, Kähkönen, Törrönen, & Heinonen, 2005; Törrönen, Kolehmainen, Sarkkinen, Mykkänen, & Niskanen, 2012). Despite of the healthiness, lingonberries are poorly utilized in food industry due to the challenging flavor characterized by high intensities of sourness, bitterness, and astringency (Laaksonen et al., 2016). These flavor characteristics are mainly caused by various phenolic compounds and organic acids (Drewnowski & Gomez-Carneros, 2000; Viljanen, Heiniö, Juvonen, Kössö, &

Puupponen-Pimiä, 2014). The most abundant classes of phenolic compounds in lingonberries are anthocyanins, flavan-3-ols, proanthocyanidins, flavonols, and phenolic acids (Kylli et al., 2011). Lingonberries contain relatively high amounts of sugars, but the high acid content masks the potential sweetness attributes (Viljakainen, Visti, & Laakso, 2002). Flavor is one of the most important attributes impacting on consumers' acceptance and consumption of food products. In the case of lingonberry, innovative approaches are needed to modify its flavor without compromising the health benefits. Sandell and co-workers (2015) showed that it is possible by modify the flavor of lingonberry by increasing the fat level and whipped airiness of the product.

Cyclodextrins (CD) are tapered cyclic oligosaccharides composed of 6, 7, or 8 α-D-glucopyranoside units (*α*-, *β*, and *γ*-CD). The inner surface of the CD cone is hydrophobic and the outer surface is hydrophilic, which enables formation of the inclusion complex with the hydrophobic molecules or the hydrophobic parts of some molecules in aqueous environment (Ayala-Zavala, Del-Toro-Sánchez, Alvarez-Parrilla, & González-Aguilar, 2008). Different cavity sizes of the CDs effect on the inclusion complexation with different sized and shaped molecules. For example, *α*-CD forms inclusion complexes typically with 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 (Ayala-Zavala et al., 2008). Also the water solubility effects on the stability of formed complex with CD: more water soluble molecules form a less stabile complex (Zhang, Cheung, Shangguan, & Zheng, 2012). Several studies have shown CDs forming 1:1 stoichiometry complexes with flavonoids (Alvarez-Parrilla, Rosa, Torres-Rivas, Rodrigo-Garcia, & González-Aguilar, 2005; Fernandes et al., 2014; L.-J. Yang et al., 2011; Zhang et al., 2012; Zhang, Jiang, Guo, & Li,

2008), but the ratio depends on structural features of the molecules such as length of carbon chains and types of functional groups (Cheirsilp & Rakmai, 2017).

CDs may potentially be used to micro-encapsulate various molecules, such as pharmaceutical substances, and to reduce undesirable tastes and odors. Micro-encapsulation also advantageously alters the chemical and physical properties of guest molecules making it more water-soluble and therefore more bioavailable (Cheirsilp & Rakmai, 2017). In food industry, CDs can potentially be used as improvers or modificators of color (Howard, Brownmiller, Prior, & Mauromoustakos, 2013) and aroma properties of food because CD can protect guest molecules from degradation during processing and storage (Andreu-Sevilla, López-Nicolás, Carbonell-Barrachina, & García-Carmona, 2011; Suvarna, Gujar, & Murahari, 2017; Szente & Szejtli, 2004). CDs are also used in green and sustainable extraction of compounds, such as phenolic compounds, from food or plant parts (Cui et al., 2017; Korompokis, Igoumenidis, Mourtzinos, & Karathanos, 2017; Ratnasooriya & Rupasinghe, 2012), and masking unfavorable taste properties, such as bitterness (Coupland & Hayes, 2014; Konno, Misaki, Toda, Wada, & Yasumatsu, 1982; Shaw, Tatum, & Wilson, 1984). When bitter molecules, such as flavonols or hydroxycinnamic acids, form the inclusion complex with CD, the bitterness of the compound is likely masked due to reduced interaction between the molecule and the bitterness receptors (Coupland & Hayes, 2014).

Gelatin is a water-soluble functional animal protein, which can form transparent gels under certain conditions (Djagny, Wang, & Xu, 2001). It is used in the wine industry as a proteinaceous fining agent due to formation of complexes with tannins, proanthocyanidins, and other phenolic compounds, which clarifies and stabilizes the wine (Waterhouse, Sacks, & Jeffery, 2016). The gelatin effects differently on proanthocyanins depending on their size: the

smaller the molecular weight of proanthocyanidin is, the less they are bound and removed (Sarni-Manchado, Deleris, Avallone, Cheynier, & Moutounet, 1999; Yokotsuka & Singleton, 1995). Anthocyanins are also bound by gelatin, which may have undesirable effect on the color of final product. (Waterhouse et al., 2016)

In this study, our aims were to investigate the impact of treatment with *β*- and *γ*- CDs and gelatin on the contents of various non-volatile components in lingonberry juice and whether use these polymers will improve the challenging flavor of lingonberry juice. Juice made from concentrate was selected as the target of this study as it is typically used in the food industry.

2 Materials and methods

2.1 Materials

Commercial lingonberry juice concentrate was purchased from Kiantama Ltd (Suomussalmi, Finland) and was stored at –20 ºC. The lingonberry juice concentrate was diluted 1:5.5 with activated charcoal filtered water to obtain equivalent of 100 % juice. *β*-CD was purchased from Roquette (Lestrem, France), and *γ*-CD was purchased from Wacker Chemical AG (Burghause, Germany).

2.2 Chemicals and standards

Hydrochloric acid was purchased from VWR International (Amsterdam, the Netherlands). Acetic acid and a 4-dimethylaminocinnamaldehyde (DMAC) reagent were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Tri-Sil® HTP reagent was purchased from Thermo Scientific (Bellefonte, PA, USA). D-sorbitol, was purchased from Fluka Biochemika (Neu-Ulm, Germany). L-(+)-Tartaric acid, L-malic acid, quinic acid, *p*-coumaric acid, 5-*O*-caffeoylquinic acid, and (+)-catechin were purchased from Sigma-Aldrich (St. Louis, MO,

USA). Quercetin-3-*O*-glucuronide was purchased from Extrasynthese (Genay, France). D-(-)-fructose and D-(+)-glucose were purchased from Merck (Darmstadt, Germany). Sucrose and citric acid were purchased from J.T. Baker Chemicals (Leuven, Belgium). Galacturonic acid was purchased from California Corporation for Biochemistry Research (Los Angeles, CA, USA). Cyanidin-3-*O*-glucoside and proanthocyanidin dimer B2 standard were purchased from Extrasynthese (Genay, France). Other HPLC and MS grade chemicals, such as methanol, acetone, ethyl acetate, acetonitrile, and formic acid were purchased from VWR international Oy (Espoo, Finland).

2.3 Preparation of lingonberry juices

Five different modified juice samples were prepared. The untreated juice was concerned as reference sample (sample Untreated). The concentration of the CDs was selected to be 10 g/L by pretesting different concentrations by a small group of panelists. This concentration corresponded to 8.8 mmol/L of *β*-CD and 7.7 mmol/L of *γ*-CD. Gelatin (sample G) treatment was performed by adding gelatin to the juice at a concentration of 1 g/L, shaking it vigorously and leaving it for 24 h at + 4 ºC. The juice was then centrifuged at 6000g for 15 min, and the supernatant was collected after centifugation. To prepare the juice treated with both gelatin and *β*-CD (sample G + *β*-CD), 10 g/L of *β*-CD was added to the supernatant. The juice with increased pH (sample pH) was prepared by the including 150 mL 1.5 mol/L sodium hydroxide (NaOH) in the dilution of the lingonberry juice concentrate to be one liter. The addition of NaOH increased the pH from 2.63 to 2.86. Untreated juice was used as a reference. All juices were prepared in triplicate and frozen at –20 °C until chemical analyses. Fresh samples were prepared for every sensory analysis.

2.4 Sensory evaluation of juices

Taste (sweetness, sourness, bitterness) and astringent properties (mouth drying and puckering) of the juices were evaluated by 40 untrained panelists (29 females, 11 males, age 19–63) in controlled laboratory conditions (ISO 8589). The attributes were defined with written examples and descriptions (sweetness: “e.g. sugar taste sweet”; sourness: “e.g. lemon taste sour”; bitterness: “e.g. grapefruit, black coffee or dark chocolate taste bitter”; mouth drying: “sample dries mouth”; mouth puckering: “sample puckers and roughens mouth”). The samples were presented in a partly fixed order: the untreated was the first sample followed by the treated juices in randomized order. The juice samples were presented as 20 mL portion in 50 mL transparent glass beakers. The panelists were asked to evaluate first the attributes from the untreated juice (labelled R) on a scale of 0–10 and then the treated juice samples (labelled with random three-digit numbers) were compared to R using a five-point category scale (1 = notably less attribute; 3 = no difference; 5 = notably more attribute). Panelists were instructed to drink water and chew a small piece of unsalted cracker between samples to clean their palates. Finally, after all the samples had been evaluated, 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).

2.5 Qualitative and quantitative analysis of sugars and organic acids

The sugars and organic acids were analyzed from the lingonberry juices with a gas chromatograph (GC-2010Plus, Shimadzu corp., Kyoto, Japan) equipped with a flame ionization detector (FID) and an AOC-20 autosampler using method described previously with slight modifications (Ma et al., 2017). The compounds were analyzed as trimethylsilyl (TMS) derivatives. External standards (glucose, fructose, malic acid, citric acid, quinic acid, and galacturonic acid) were used to identify the main sugars and organic acids. Sorbitol and tartaric

186 acid (both 0.5 g/100 mL) were used as internal standards for quantification of sugars and
187 organic acids, respectively. Two hundred and fifty μL of juice and both internal standards were
188 mixed, and the mixture was diluted to final volume of 5 mL with ultra-pure water (Millipore
189 Corporation, Darmstadt, Germany). The sample was then filtered with a regenerated cellulose
190 syringe filter (0.45 μm). A portion of 300 μL of the filtrate was pipetted to an autosampler vial
191 and evaporated to dryness at 50 °C under a nitrogen flow. the samples were stored in the
192 desiccator until analysis at least overnight. Five hundred μL of Tri-Sil reagent
193 (hexamethyldisilazane:trimethylchlorosilane:pyridine, 2:1:10, Thermo Scientific, Pierce
194 Biotechnology, Rockford, IL, USA) was added to the samples for trimethylsilyl (TMS)
195 derivatization; the sample was then vortexed vigorously and incubated for 30 min at 60 °C.
196 The analyses of the derived samples were carried out with Supelco Simplicity-1 fused silica
197 column (30 m × 0.25 mm i.d. × 0.25 μm d_f, Supelco, Bellefonte, PA, USA). Analysis conditions
198 were the same as previously reported by Ma et al. (2017). Analyses were carried out in
199 triplicate.
200
201 2.6 Purification of proanthocyanidins
202 Purification of proanthocyanidins (PAs) was accomplished as described previously (W. Yang,
203 Laaksonen, Kallio, & Yang, 2016) with column chromatograph packed with five grams of
204 Sephadex LH-20 (Pharmacia, Uppsala, Sweden). The flow rate was maintained at 1 mL/min
205 with an Alite-XV peristaltic pump (Bioengineering, Wald, Switzerland). Solvents in the PA
206 fraction were removed with a vacuum rotary evaporator at 40 ºC (Heidolph Instruments GmbH
207 & Co. KG, Schwabach, Germany). The PAs were dissolved in 1 mL of methanol and filtered
208 through a 0.22 μm polytetrafluoroethylene (PTFE) filter (VWR International, Allison Park,
209 PA, USA). Two purified samples were prepared from all lingonberry juices.
210

2.7 Identification of proanthocyanidins

Identification of the PAs was carried out with UPLC-MS. The instrument used was a Waters Acquity ultrahigh performance LC system (Waters Corp., Milford, MA, USA), which consisted of a sample manager, a binary solvent delivery system, and a Waters 2996 PDA detector. The compounds were separated on a Phenomenex Luna HILIC 200A column (3 µm, 150 x 3.00 mm, Phenomenex, Torrance, CA, USA). The LC was linked to a Waters Quattro Premier tandem quatropole mass spectrometer (Waters Corp., Milford, MA, USA) equipped with an electrospray ionization (ESI) source. The instrument was operated using MassLynx 4.1 software (Waters Corp., Milford, MA, USA). The instrument parameters and eluents were same as before reported by Kallio, Yang, Liu, and Yang, (2014).

Both full scan and selected ion recording (SIR) functions were used. The total ion ESI-MS analysis was carried out by scanning ions from *m/z* 250 to 3000 for oligomer size screening. The *m/z* signals chosen for the SIR were the most abundant deprotonated PA ions calculated based on the nominal masses.

2.8 Total proanthocyanidin content

The total PA content was determined spectrophotometrically using the Brunswick Laboratories 4-dimethylaminocinnamaldehyde (BL-DMAC) assay and an external standard as a description given previously (W. Yang et al., 2016).

2.9 Analysis of phenolic acid derivatives, flavonol glycosides and anthocyanins

Phenolic acid derivatives, flavonol glycosides and anthocyanins were isolated and quantified with HPLC-DAD as described previously (Laaksonen et al., 2012) using Shimadzu's Nexera 30-series UHPLC system coupled with SIL-30AC autosampler, two LC-30AD solvent pumps, CTO-20AC column oven, SPD-M20A diode array detector (DAD), and CBM-20A

communication unit (Kyoto, Japan). Phenolic acids were recorded at 310 nm 320 nm, and flavonols at 360 nm. The juice samples were analyzed in duplicates. *p*-Coumaric acid, 5-*O*-caffeoylquinic acid, and quercetin-3-*O*-glucuronide were used for quantification of the compounds recorded at 310, 320, and 360 nm, respectively. A five-point calibration curve was constructed in the concentration range of 0.0050–0.50 mg/mL. The standard solutions were analyzed in duplicate, and the mean values were used to draw the calibration curves.

Anthocyanins were analyzed with the same HPLC-DAD instrument and column as described previously (Tian et al., 2017) with slight modifications. Before analysis, 500 µL of the juice samples were diluted with 500 µL of acidified methanol and filtered through a 0.22 µm polytetrafluoroethylene (PTFE) filter. The mobile phases were water (A) and acetonitrile (B), both containing 50 mL/L of formic acid. The following gradient was used for the mobile phase B: 0–10 min, 5–10 %; 10–20 min, 10–25 %; 20–30 min, 25–90 %; 30–35 min, 90–5 %. The column temperature was 36 ºC, and the peaks were recorded at 520 nm.

Identification of the anthocyanins and other phenolic compounds was carried out with the same UPLC-DAD-ESI-MS instrument as previously mentioned. The column, eluents and gradient programs were the same as in HPLC-DAD analysis. Before MS analysis, the samples were concentrated to 4-fold. A flow of 0.5 mL/min was directed to the mass spectrometer operated in the positive- or negative-ion mode. The capillary voltage was 3.51 kV, the cone voltage 15 V, and the extractor voltage 3 V. The source temperature was 120 °C, and the desolvation temperature was 300 °C. The desolvation gas flow was 599 L/h, and the cone gas flow was 97 L/h. Mass spectra were obtained by scanning ions between *m*/*z* 130 and 880.

2.10 Statistical analysis

All juices were produced in triplicate, and measurements were carried out in duplicate or triplicate. Results were expressed as means ± SD (standard deviation). Statistical analyses were performed using IBM SPSS software version 24 (IBM Corp., Armonk, NY). Difference between treatments was analyzed using one-way ANOVA with post-hoc analysis using the HSD Tukey test or Tamhane's test. The sensory data from the five-point category scale was transformed to 0/1 data and analyzed using McNemar's test. Compositional profiles of the juice samples were also investigated using Principal Component Analysis (PCA) with standardized data using Unscrambler X (version 10.4, CAMO Inc, Oslo, Norway)

3 Results and discussion

3.1 Chemical composition and quantitative description of lingonberry juice

A total of 52 non-volatile compounds were identified or preliminarily identified from the lingonberry juice, of which forty-five were phenolic compounds (phenolic acids and their derivatives, flavanols, flavonols, and anthocyanins) commonly associated with a bitter taste and an astringent mouthfeel, and four organic acids (sourness) and two sugars (sweetness).

Twenty-eight compounds were quantified. The acids and sugars detected from the lingonberry samples were in accordance with the literature (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Veberic, 2012; Viljakainen et al., 2002). Quinic acid and citric acid were the most abundant acids in the lingonberry juices (**Table 1**). Other organic acids detected were malic acid and galacturonic acid. Galacturonic acid is a sugar acid and main monomeric unit in the berry pectins, released from pectin by pectinolytic enzymes, which are used in the food industry to increase the yield of juice during juice processing. Free galacturonic acid is not found in fresh lingonberries. Galacturonic acid may potentially affect the sourness of a food product, if the concentration is over its detection threshold. Hufnagel and Hofmann (2008) determined the

taste threshold for galacturonic acid to be 0.643 mmol/L. In the lingonberry juice samples, galacturonic acid concentrations varied between 11.3 and 11.8 mmol/L, which are significantly higher than the reported threshold. Mikulic-Petskovsek et al. (2012) also detected tartaric acid, fumaric acid, and sucrose in lingonberry berries, but these were not found in the juice concentrate used in this study. The only sugars detected were glucose and fructose, being present at almost equal concentrations. Contents of organic acids and sugars are in line with or lower than in the fresh lingonberry berries (Mikulic-Petkovsek et al., 2012; Viljakainen et al., 2002), which might have been due to the concentrating of the juice. In this study, all the comparison is done to the fresh berries, because data about lingonberry juices was not found.

Laaksonen et al. (2013) reported that enzymatically assisted juice processing of black currant juice caused a decrease in the sugar/acid ratio, and the low sugar/acid ratio correlated positively with high intensities of sourness and mouth-drying astringency. In our study, the sugar/acid ratio of the lingonberry juice concentrate was 2.27, whereas Mikulic-Petskovsek et al. (2012) reported pH value of 2.72 for the unprocessed lingonberry juice. Thus the juice made from the juice concentrate used in our study may potentially be even more sour and astringent and less sweet than the fresh berries used in the study of Mikulic-Petskovsek et al (2012).

Three typical anthocyanins for lingonberry were identified using UV spectra, reference standards, and the literature (Ek, Kartimo, Mattila, & Tolonen, 2006). The most abundant was cyanidin-3-*O*-galactoside (13.8 mg/100 mL) and the others were cyanidin-3-*O*-glucoside (2.5 mg/100 mL) and cyanidin-3-*O*-arabinoside (4.0 mg/100mL). The total concentration of anthocyanins was 20.2 mg/100 mL (**Table 1**). According to Dróżdż et. al (2017) and Lee and Finn (2012) the total concentrations of anthocyanin in lingonberry varieties are between 26.1 and 51.6 mg/100 g of fresh berry weight, which is higher than in our juice. Anthocyanins are

relatively unstable and they may be degraded by, for example, an increase of pH or temperature and by the presence of oxygen (Castañeda-Ovando, Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009), which can easily explain the difference between our data and the reporter data.

Phenolic acids and their derivatives were tentatively identified with UHPLC-DAD-MS (**Fig. 1, Table 2**). A total of 10 phenolic acids and derivatives were tentatively identified. To our knowledge, vanillic acid hexoside (λ_{max} 323 nm, $[M-H]^-$ *m*/*z* 329) was identified for the first time in lingonberry juice, although, Mattila *et al.* (2006) identified vanillic acid in lingonberry. Otherwise, the phenolic acid derivatives identified were in line with the literature (Ek et al., 2006; Hokkanen, Mattila, Jaakola, Pirttilä, & Tolonen, 2009; Ieri, Martini, Innocenti, & Mulinacci, 2013; Mattila, Hellström, & Törrönen, 2006; Seraglio et al., 2018; Tian et al., 2017). The most abundant phenolic acid derivatives were 5-*O*-caffeoylquinic acid (chlorogenic acid, 4.7 mg/100mL), ferulic acid derivative (3.0 mg/100 mL) and *p*-coumaric acid (2.4 mg/100 mL), respectively (**Table 1**). The total amount of phenolic acids and their derivatives was 17.1 mg/100 mL of lingonberry juice. *p*-coumaric acid is an astringent compound with a detection threshold 139 μmol/L (Hufnagel & Hofmann, 2008). The concentration in our lingonberry juice corresponds to 143.3 μmol/L, thus affecting the astringency.

Eleven flavonols were tentatively identified with UHPLC-DAD-MS and the literature (**Table 2, Fig. 1**) (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). All identified flavonols were 3-*O*-glycosides of quercetin and the free aglycon potentially released from the glycoside structures during the juice processing. To our knowledge, our study identified quercetin glucuronide for the first time in lingonberry juice. Kaempferol glycosides have been reported in the lingonberries

previously (Antolak et al., 2017; Ek et al., 2006; Lehtonen, Lehtinen, Suomela, Viitanen, & Kallio, 2010). The most abundant flavonols were quercetin rhamnoside (11.8 mg/100 mL), quercetin aglycon (9.7 mg/100 mL), quercetin-3-*O*-4"-(3-hydroxy-3-methylglutaroyl)-rhamnoside (7.3 mg/100 mL), and quercetin galactoside (5.7 mg/100 mL). Lehtonen et al. (2010) also found quercetin rhamnoside to be the main flavonol glycoside in lingonberries; the contents of flavonols reported in their study were notably lower than the levels found in our study. Häkkinen, Kärenlampi, Heinonen, Mykkänen, and Törrönen, (1999) reported the total flavonol content of fresh lingonberries to be between 74–146 mg/kg, which is notably higher than ours (46.2 mg/100 mL).

Twenty peaks were detected as proanthocyanidins (PAs) from the UV spectra recorded at 280 nm (**Fig. 2A**). However, it was not possible identify individual PAs from the chromatogram, because the peaks overlapped. Full scan (*m/*z from 250 to 3000) ESI-MS spectra were used to tentatively identify the oligomer profile of the PAs. The total ion spectrum is shown in **Fig. 2B**. PAs with the degree of polymerization (PA) between 1 and 9 were identified. Both A-type and B-type PAs with DP values of 2, 3, 4, 5, and 9 were identified. Only B-type PAs were identified with DP values of 6, 7, and 8. Both A- and B-type PAs dimers and trimers have been reported in lingonberries (Jungfer, Zimmermann, Ruttkat, & Galensa, 2012). The $[M - H]^-$ ions of PAs of DP from 1 to 6, $[M - 2H]^{2-}$ ions of PAs of DP from 7 to 9 and the linkage types between the subunits are presented in the **Table 3**. The total content of PAs was analyzed using spectrophotometry with a (DMAC) reagent. For reference, in the lingonberry juice the total amount of PAs was 109.4 mg/100 mL quantified as equivalent procyanidin B2. Previous studies have reported the total concentration of proanthocyanins to vary between 260 and 350 mg/100 g of fresh weight of lingonberries (Grace, Esposito, Dunlap, & Lila, 2014; Hellström, Törrönen, & Mattila, 2009; Kylli et al., 2011). The notably lower levels of flavonols and PAs

359 in the juice than the levels previously reported in the berries was most likely due to the juice
360 processing, such as pressing, fining procedure, and concentrating process, where some of the
361 compounds are removed by precitpitation (fining) or they can be degraded by effect of heating
362 (Elik, Yanık, Maskan, & Göğüş, 2016; Waterhouse et al., 2016).
363
364 3.2 Changes in chemical compositions
365 Principal component analysis model was created with 26 chemical variables and six juice
366 samples. PCA model with first three principal components are shown in **Fig. 3** showing in total
367 87 % of the variation in the data. Generally, the gelatin treatment (G) decreased the content of
368 the majority of the phenolic compounds (located on the left on the PC1) whereas addition of
369 the *β*-CD (*β*-CD) resulted in increased contents, especially flavonols (on the right on the PC1).
370 *γ*-CD (*γ*-CD) did not have a similar effect, which may have resulted from slightly different
371 molar concentration used for the *γ*-CD. Treatments did not significantly have impact on the
372 concentrations of sugars and organic acids, because sugars and organic acids are highly water
373 soluble which inhibits the formation of inclusion complex with CDs. The second component
374 showed the correlations between G+*β*-CD sample and some of the phenolic acids whereas the
375 third component separated the untreated original juice from the *β*-CD with more fruit acids,
376 cyanidin glucoside and phenolic acids in the latter sample.
377
378 None of the treated samples differed statistically from the untreated juice sample in the content
379 of anthocyanins (**Table** 1). However, there were significant differences in the concentrations
380 of anthocyanins among the treated samples, the levels in the CD treated juices being higher
381 than those in the samples treated with gelatin, G+*β*-CD, and pH increase (**Table** 1). Previously,
382 CDs have been shown to protect anthocyanins and other food components effecting on sensory
383 quality from degradation caused by thermal processing and storing (Fernandes et al., 2018;

384 Howard et al., 2013; Szente & Szejtli, 2004), which could explain the higher anthocyanin
385 contents in the CD samples.
386
387 The effects of gelatin treatment on hydroxycinnamic acids were scarce on statistical level,
388 which is consistent with results published by Hubert, Baron, Le Quere, and Renard (2007) and
389 Cosme, Ricardo-da-Silva, and Laureano (2008). Gelatin treatment lowered the content of 3-*O*-
390 caffeoylquinic acid compared with other treatments and the non-treated juice. Gelatin treatment
391 also decreased the *p*-coumaric acid content. *p*-Coumaric acid is known as a potential
392 contributor to astringency (Hufnagel & Hofmann, 2008). After the gelatin treatment, the level
393 of *p*-coumaric acid was below the taste threshold reported for the compound (139 μmol/L)
394 (Hufnagel & Hofmann, 2008), therefore the treatment might have an effect on the astringency
395 potentially caused by the *p*-coumaric acid. CD treatments had also only small effect on the
396 hydroxycinnamic acids and their derivatives. CDs inclusion complex forming effectivity is
397 known to be affected by shape, size, and water solubility of guest molecule. Cavity of γ-CD is
398 too big for some of the hydroxycinnamic acids, such as ferulic acid. When the cavity is too
399 wide and guest molecule too small the interactions cannot happen and the complex is not
400 formed (Zhang et al., 2012).
401
402 Gelatin treatment decreased again the concentrations of all flavonol compounds compared to
403 untreated juice, but this was only statistically significant for quercetin xyloside,
404 arabinofuranoside, and quercetin aglycone (**Table 1**). At the same time, the similar protective
405 phenomenon was observed with CDs as in anthocyanins as the β-CD increased concentrations
406 of some flavonols compared to untreated juice, but the increase was not significant. The most
407 significant and abundant differences were detected between β-CD and gelatin treatments,
408 including some of the most abundant quercetin glycosides in the samples. Moreover,

409 significant differences were also detected in some of the flavonols (quercetin glucuronide,
410 xyloside, arabinoside, arabinofuranoside, and quercetin aglycone) between gelatin treated and
411 G+β-CD treated samples. The only flavonols not statistically affected by any of the treatments,
412 were quercetin glucoside and quercetin rhamnoside.
413
414 The total amounts of proanthocyanidins varied between 81.72 and 114.16 mg/100 mL.
415 Treatments did not effect on PA concentrations with statistical significance, but the gelatin
416 treatment has lowering effect on the amount of total PAs. Concentrations of individual PAs
417 were not measured, so it cannot be said which PAs were removed with gelatin. CDs had only
418 little effect on the PAs. It can be caused by the shape or size of the proanthocyanidins making
419 them unable to unfit inside CD cavities.
420 Previous studies have reported that better phenolic compound recoveries and more stable
421 complexes with different CDs. Ratnasooriyav and Rupasinghe (2012) reported that ferulic acid
422 recovery to be higher with α-CD than with β-CD, and for chlorogenic acid recovery was higher
423 with β-CD. Zhang et al. (2012) reported astilbin and taxifolin form the most stabile complexes
424 with β-CD, when compared to α- and γ-CDs. L.-J. Yang et al. (2011) reported both β- and γ-
425 CD are suitable to form complexes with taxifolin and concluded that size-fit effect is the
426 dominant controlling factor on the inclusion complex between taxifolin and CD. All these
427 results indicate that the most effective way to encapsulate more phenolic compounds with CDs
428 is to use mixture of CDs.
429
430 3.3 Sensory quality of the lingonberry juices
431 The sensory quality (taste and astringency) of the 100 % lingonberry juice prepared from the
432 concentrate was rated on a scale from 0–10, and the results are shown in **Table 4**. All the juices
433 were evaluated as notably sour, bitter and astringent with very little sweetness. The untreated

juice was then compared to the treated samples, which were presented in a randomized order. The data is shown in **Table 4** as frequencies of participants who detected an increase in sweetness or a decrease in sourness, bitterness or astringent properties in treated juices as compared to the untreated. Interestingly, the differences were detected more often in the juices with two treatments (β-CD and gelatin treatment or higher pH) in comparison to the juices with only one treatment (e.g. gelatin treated or CD). This indicates that treatment with CDs alone may not be sufficient alone to affect the negative sensory attributes. The used concentration of CDs (10 g/L) in this study was selected to be above the levels used by Konno, Misaki, Toda, Wada, and Yasumatsu (1982), and Gaudette and Pickering (2012). In studies by Gaudette et al. (2016, 2012a) β-CD alone did not reduce the bitterness of (+)-catechin whereas bitterness was significantly reduced by combined treatment with CD and sweeteners. However, the efficacy of the bitter blockers is highly dependent on the bitter compound (Gaudette & Pickering, 2012b). β-CD has been reported to successfully masking the bitter taste in concentrates as low as 3 g/L in grape fruit and mandarin juices (Szente & Szejtli, 2004).

Despite the high concentrations of various known bitter compounds, the key contributors to the bitterness in lingonberries have not been reported. Here, the juice sample first treated with gelatin and later supplemented with β-CD, was more often perceived different in mouth-drying astringency than other modified juices when the sample was compared to the original juice in that particular attribute (**Table 4**). The treatments with gelatin and CD showed some synergistic effects. The gelatin may have first removed some components from the juice which may have enabled the CD to block others contributors of astringency. At the end of the sensory test, participants were asked to select the most and the least preferred samples among the juices (**Table 4**). The juices with two treatments were more often picked as the most liked and less

458 frequently as the least liked samples compared to other juices, indicating significant changes
459 in the sensory profile towards more pleasant direction.
460
461 Nevertheless, in this study the addition β-CD did not have notable effect on the bitter taste or
462 astringency of the lingonberry juice. However, the key compounds responsible for the
463 bitterness and astringency in lingonberry are not known despite the fact that berry contains
464 several astringent and bitter compounds. Moreover, blocking (CDs) or removing (gelatin) some
465 of the key compounds for one taste attribute may result in emphasis of other taste attributes,
466 e.g. blocking of lingonberry bitterness may emphasize sourness or other main taste
467 characteristics of the berry. Ultimately, consumers often prefer natural flavors of berries.
468 Therefore, intensive modification of the original berry composition may result in altered
469 sensory qualities reduced consumer acceptance

470 **4 Conclusions**

471 For our knowledge, this was the first research of effects of the β- and γ-cyclodextrins on the
472 phenolic compounds and sensory properties of lingonberry juice diluted from concentrate. At
473 molecular level, gelatin treatment decreased the content of phenolic compounds, whereas
474 cyclodextrin, especially β-cyclodextrin treatment tended to increase the content of phenolic
475 compounds, suggesting protective effects of cyclodextrin on these compounds from
476 degradation during juice processing. β-cyclodextrin was used in the sensory evaluation, but
477 itself did not have significant effect on the sensory properties of lingonberry juice even though
478 the use concentration was higher than used in previous studies. When gelatin treatment and β-
479 cyclodextrin treatment were used sequential, the resulting juice was the most pleasant for the
480 panelists. In this study, intensive bitterness and astringency limited the sample amount in the
481 sensory evaluation and only one concentration of β-cyclodextrin was evaluated. The unwanted
482 sensory characteristics can be affected by removing the compounds; however, this may

compromise the health benefits of the juices since phenolic compounds have been known to have various beneficial effects on human health. Further optimizations and alternative methodologies are needed to successfully modify the unwanted sensory characteristics of without affecting its healthiness or unique original flavor. This study provides important information about the use of CDs for modifying the sensory quality of juices rich in phenolic compounds.

Acknowledgements

We acknowledge Mr. Niko Markkinen for technical support. This work was supported by Ab Fazer Oy, TOP foundation and Turku University Foundation, Finland.

Conflict of interest statement

Authors declare no conflicts of interest.

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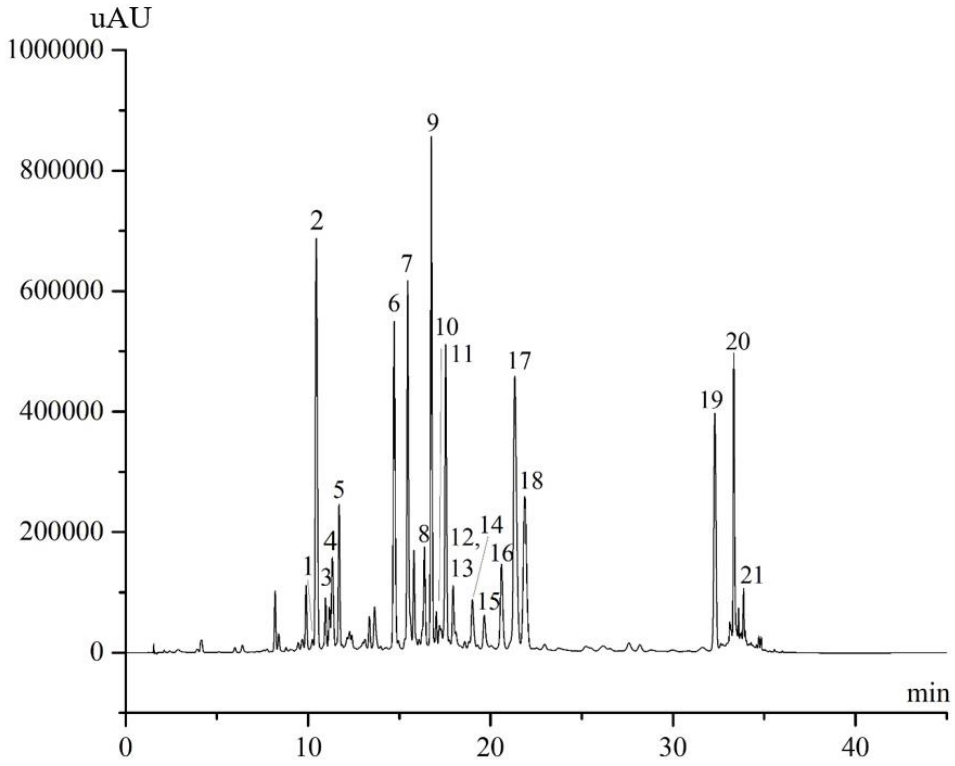
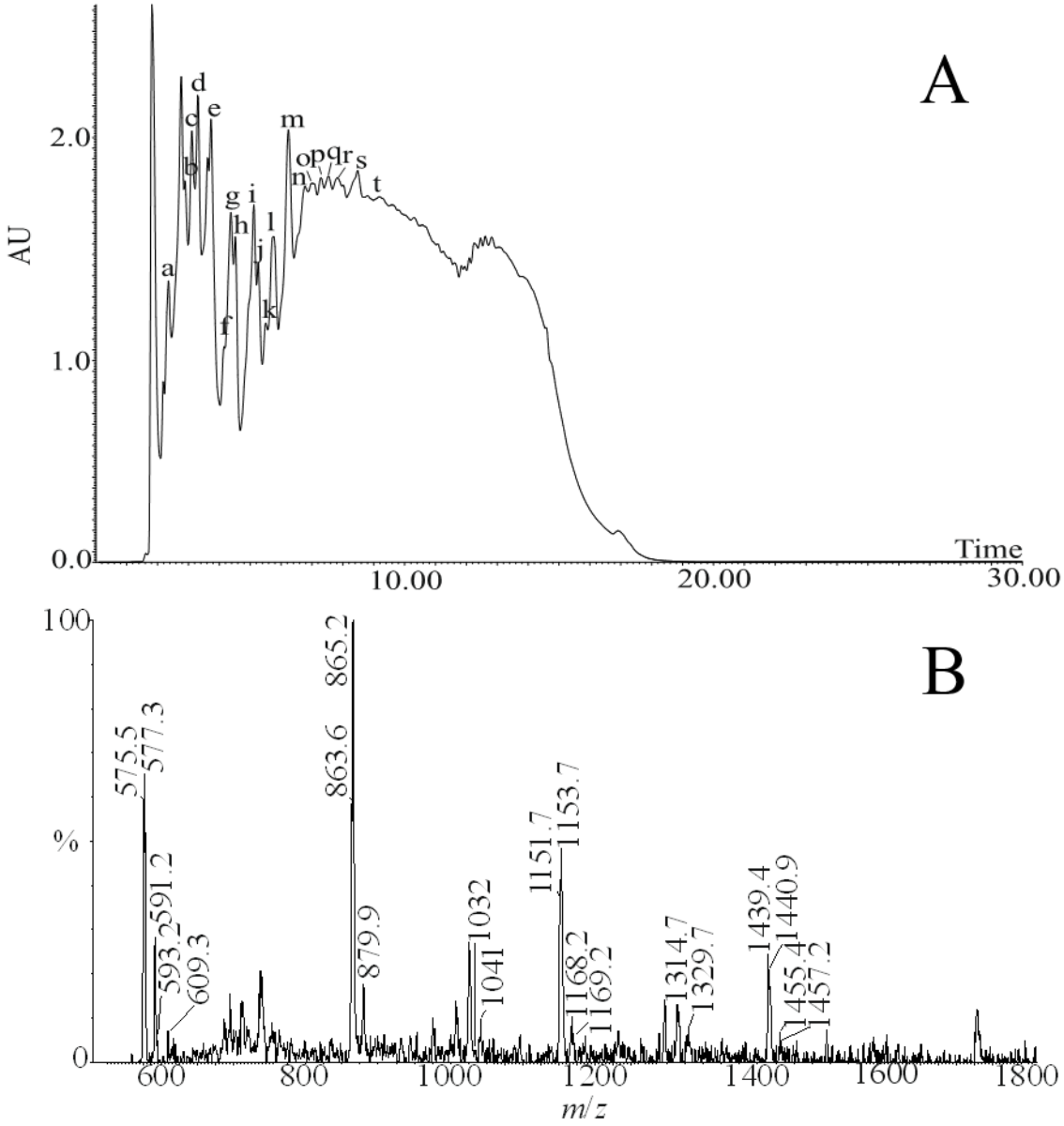


718

719 Fig. 1 HPLC chromatogram of phenolic acids and flavonols in an ethyl acetate extract of

720 lingonberry juice. Numbers reference to the Table 2.

721

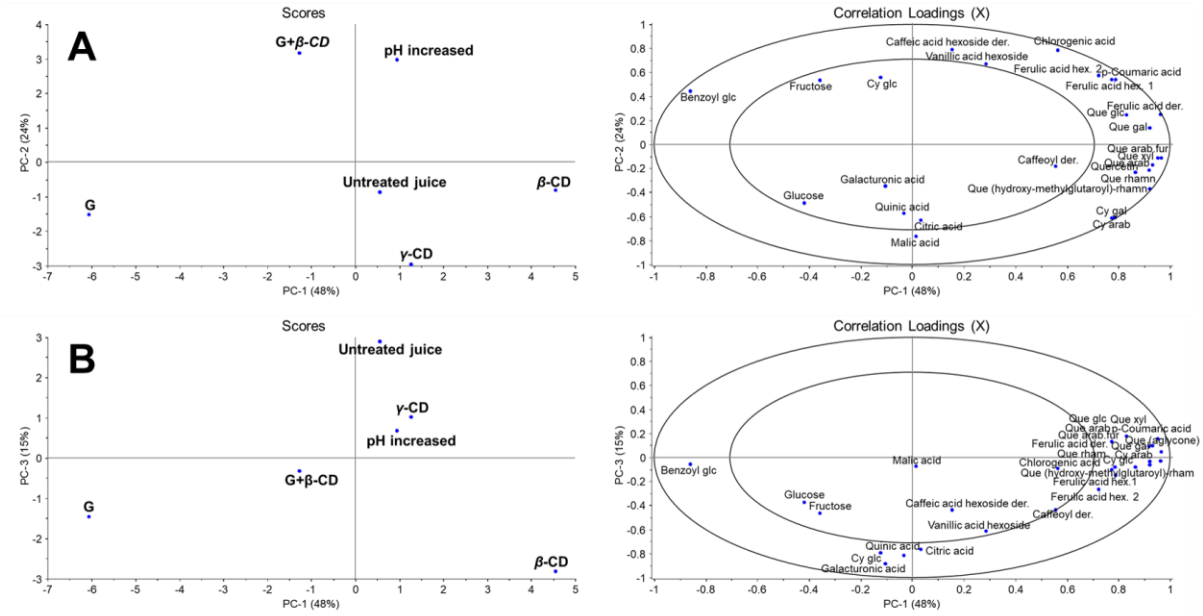


722

723 Fig. 2 HILIC-HPLC chromatogram of proanthocyanins in the lingonberry juice recorded at

724 280 nm (A) and ESI-MS total ion spectrum of proanthocyanidins in the lingonberry juice (B)

725 from 3.0 to 18.0 min in the chromatogram.



726

727 Fig. 3 . Principal component analysis model based on the chemical variables (n = 26, blue) in
728 the lingonberry juice samples (n = 6, green). A. Scores and loading plots with PC1 and PC2;
729 B. PC1 and PC3. Abbreviations: β-CD β-cyclodextrin; γ-CD γ-cyclodextrin; G gelatin; G+β-
730 CD sequential treatment with gelatin and β-cyclodextrin.

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Table 1 Concentrations of organic acids, sugars, anthocyanins, phenolic acid derivatives, flavonols and total proanthocyanidins (mg/100 mL) in the commercial lingonberry concentrate diluted correspond to 100 % juices before and after treatments.

	Untreated juice	*β*-CD	*γ*-CD	G	G + *β*-CD	pH increased
Organic acids						
Malic acid	42.1 ± 0.8	42.8 ± 1.3	44.8 ± 6.0	43.1 ± 0.6	42.2 ± 0.7	42.1 ± 0.7
Citric acid	1 457 ± 40	1 496 ± 39	1 476 ± 31	1 489 ± 21	1 457 ± 29	1 456 ± 29
Quinic acid	1 494 ± 148	1 528 ± 167	1 509 ± 154	1 523 ± 135	1 498 ± 130	1 492 ± 130
Galacturonic acid	218.7 ± 7.7	224.9 ± 5.1	220.7 ± 5.6	224.7 ± 3.4	219.7 ± 7.2	220.5 ± 7.2
Total	3211.3 ± 200.0	3291.7 ± 217.5	3251.4 ± 193.4	3279.6 ± 144.9	3216.3 ± 170.7	3210.9 ± 141.2
Sugars						
Fructose	3 636 ± 171	3 771 ± 308	3 671 ± 188	3 882 ± 294	3 782 ± 327	3 938 ± 327
Glucose	3 661 ± 154	3 692 ± 192	3 702 ± 191	3 754 ± 197	3 722 ± 285	3 535 ± 285
Total	7297 ± 102.9	7463 ± 248.6	7373 ± 176.5	7636 ± 43.1	7504 ± 276.8	7473 ± 160.9
Anthocyanins						
Cyanidin-3-*O*-galactoside	13.75 ± 0.59ab	14.26 ± 0.47b	14.21 ± 0.13b	13.37 ± 0.30a	13.34 ± 0.40a	13.54 ± 0.40a
Cyanidin-3-*O*-glucoside	2.49 ± 0.11ab	2.58 ± 0.07b	2.56 ± 0.03b	2.42 ± 0.06a	2.42 ± 0.07a	2.43 ± 0.07a
Cyanidin-3-*O*-arabinoside	3.99 ± 0.18ab	4.14 ± 0.11b	4.12 ± 0.04b	3.86 ± 0.10a	3.85 ± 0.12a	3.92 ± 0.12a
Total	20.2 ± 0.83	21.0 ± 0.54	20.9 ± 0.76	19.7 ± 0.45	19.6 ± 0.38	19.9 ± 0.57
Hydroxycinnamic acids						
Caffeic acid-hexoside derivative	0.31 ± 0.04	0.33 ± 0.00	0.32 ± 0.02	0.32 ± 0.02	0.34 ± 0.01	0.33 ± 0.01
5-*O*-caffeoylquinic acid (chlorogenic acid)	4.70 ± 0.19ab	4.79 ± 0.09ab	4.56 ± 0.07ab	4.53 ± 0.19a	4.80 ± 0.13b	4.80 ± 0.13b

1-*O*-Benzoyl-beta-glucose	0.21 ± 0.01a	0.20 ± 0.01a	0.21 ± 0.01a	0.24 ± 0.02b	0.23 ± 0.02ab	0.23 ± 0.02ab
Ferulic acid hexoside 1	1.11 ± 0.03ab	1.15 ± 0.03b	1.10 ± 0.01ab	1.06 ± 0.04a	1.14 ± 0.03b	1.13 ± 0.03b
Vanillic acid hexoside	1.19 ± 0.12a	1.31 ± 0.03bc	1.21 ± 0.04ab	1.22 ± 0.04abc	1.33 ± 0.04abc	1.27 ± 0.04abc
p-coumaric acid	2.35 ± 0.07b	2.37 ± 0.04b	2.30 ± 0.02ab	2.22 ± 0.04a	2.37 ± 0.02b	2.35 ± 0.02b
2"-*O*-caffeoylarbutin	0.99 ± 0.06abc	1.23 ± 0.10c	1.05 ± 0.03bc	0.90 ± 0.05a	1.11 ± 0.38abc	0.83 ± 0.38abc
Ferulic acid hexoside 2	2.30 ± 0.07ab	2.43 ± 0.04b	2.35 ± 0.03ab	2.25 ± 0.11a	2.41 ± 0.02b	2.42 ± 0.02b
Ferulic acid derivative	3.01 ± 0.08b	3.18 ± 0.05b	3.01 ± 0.05b	2.73 ± 0.20a	3.02 ± 0.08b	3.06 ± 0.08b
Total	17.05 ± 0.43	17.91 ± 0.29	17.00 ± 0.21	16.26 ± 0.72	17.64 ± 0.5	17.30 ±0.32
Flavonols						
Quercetin-3-*O*-galactoside	5.74 ± 0.09ab	5.86 ± 0.16b	5.70 ± 0.13ab	5.56 ± 0.08a	5.64 ± 0.13ab	5.81 ± 0.13ab
Quercetin-3-*O*-glucuronide	1.67 ± 0.07ab	1.72 ± 0.09b	1.74 ± 0.08b	1.54 ± 0.09a	1.72 ± 0.04b	1.71 ± 0.04b
Quercetin-3-*O*-glucoside	0.56 ± 0.03	0.59 ± 0.06	0.52 ± 0.04	0.50 ± 0.04	0.57 ± 0.07	0.53 ± 0.07
Quercetin-3-*O*-xyloside	1.32 ± 0.02bc	1.35 ± 0.04c	1.33 ± 0.03bc	1.24 ± 0.02a	1.27 ± 0.04bc	1.33 ± 0.04bc
Quercetin-3-*O*-arabinoside	1.57 ± 0.04abc	1.63 ± 0.04c	1.63 ± 0.08c	1.48 ± 0.04a	1.53 ± 0.04bc	1.60 ± 0.04bc
Quercetin-3-*O*-arabinofuranoside	3.59 ± 0.05bc	3.71 ± 0.07c	3.60 ± 0.05bc	3.37 ± 0.09a	3.45 ± 0.09bc	3.61 ± 0.09bc
Quercetin-3-*O*-rhamnoside (STD)	11.77 ± 0.17ab	12.11 ± 0.15b	11.86 ± 0.15ab	11.47 ± 0.34a	11.42 ± 0.33ab	11.91 ± 0.33ab
Quercetin-3-*O*-4"-(3-hydroxy-3-methylglutaroyl)-rhamnoside	7.32 ± 0.17abc	7.56 ± 0.17c	7.38 ± 0.17bc	6.98 ± 0.08a	7.09 ± 0.27ab	7.19 ± 0.27ab
Quercetin (agl)	9.74 ± 0.30c	10.02 ± 0.18c	9.49 ± 0.32bc	8.78 ± 0.12a	8.97 ± 0.54bc	9.48 ± 0.54bc
Quercetin-3-*O*-(6"-benzoyl)-galactoside	2.95 ± 0.13b	2.95 ± 0.06b	2.90 ± 0.07b	2.56 ± 0.17a	2.55 ± 0.25a	2.91 ± 0.25b
Total	46.23 ± 0.72	47.50 ± 0.41	46.15 ± 0.91	43.48 ± 0.63	44.21 ± 1.69	46.08 ± 1.74

Total proanthocyanidins with DMAC	109.40 ± 23.33	98.51 ± 14.34	114.16 ± 44.88	81.72 ± 16.63	82.20 ± 23.57	101.56 ± 27.46

All concentrations are mean values ± SD of 2-3 analytical replicates from triplicate samples. Samples with the same letters for each attribute do not differ significantly (One-way ANOVA and Tukey's test, $p < 0.05$).

Table 2 Phenolic acid derivatives and flavonols tentatively identified in the commercial lingonberry concentrate diluted correspond to 100 % juice and their RP-UPLC-MS data.

Peak#	Compound	λ_{max} (nm)	$[M+H]^+$ (*m/z*)		$[M-H]^-$ (*m/z*)		References
	Phenolic acid derivatives		Nominal masses	Ion source fragments	Nominal masses	Ion source fragments	
1	Caffeic acid hexoside	313	343		341		[1]
2	5-*O*-caffeoylquinic acid (chlorogenic acid)*	319	355				[1, 3]
3	1-*O*-Benzoyl-beta-glucose	275	285	569			[1]
4	Vanillic acid hexoside	323			329		[1]
5	Ferulic acid hexoside	326	357	195			[1, 5]
6	*p*-coumaric acid*	310	165		163		[1, 2]
7	Ferulic acid hexoside	320	357	195	355		[1]
8	2''-*O*-caffeoylarbutin	322	435	325			[4, 6, 5]
9	Ferulic acid hexoside	313	357	195	355	192	[1, 5]
18	Ferulic acid derivative	325			387	193	[1, 2]
	Flavonols						
10	Quercetin 3-*O*-rutinoside		611				[1, 4, 5]
11	Quercetin 3-*O*-galactoside	354	465				[1, 4, 5]
12	Quercetin 3-*O*-glucuronide*	351	479		477		[1, 4]
13	Quercetin 3-*O*-glucoside*	354	465		463		[1, 4, 5]
14	Quercetin 3-*O*-xyloside	354	435		433		[1, 4, 5]
15	Quercetin 3-*O*-arabinoside	354	435		433		[1, 4, 5]
16	Quercetin 3-*O*-arabinofuranoside	354	435		433		[1, 4, 5]
17	Quercetin 3-*O*-rhamnoside*	346	449		448		[1, 4, 5]

19	Quercetin 3-*O*-4''-(3-hydroxy-3-methylglutaroyl)-rhamnoside	346	593		591	[1, 4, 5]
20	Quercetine (aglycone)	368	303		301	[1, 2, 4]
21	Quercetin 3-*O*-(6″-benzoyl)-galactoside	355	569	303	567	[1]

*Identification is made also with the external standard. [1]Tian et al., 2017; [2]Seraglio et al., 2018; [3]da Silveira et al., 2017; [4]Hokkanen, Mattila, Jaakola, Pirttilä, & Tolonen, 2009; [5]Ek et al., 2006 [6]Ieri, Martini, Innocenti, & Mulinacci, 2013.

Table 1 Proanthocyanidins identified in lingonberry juice and their MS data.

		Number 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			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 polymerization, (E)C (epi)catechin, (E)GC gallocatechin

Table 4. Sensory profile of lingonberry juice made from concentrate, the frequencies of untrained panelists detecting differences in the modified juice samples in comparison to the untreated juice and frequencies (%) of most and least liked juice samples

Juice samples	Sweetness	Sourness	Bitterness	Mouthdrying astringency	Puckering astringency	Most pleasant sample (%)	Least pleasant sample (%)
Untreated [a]	1.93 ± 1.6	7.3 ± 1.4	6.75 ± 2.0	5.85 ± 2.2	5.68 ± 2.3	0	32.5
Comparison to the untreated [b]	more sweet	less sour	less bitter	less mouthdrying	less puckering		
Gelatin (G)	8 c	13 c	15 b	18 b	15 c	17.5	15
Cyclodextrin (β-c)	21 ab	17 abc	17 b	17 b	21 bc	12.5	15
pH treated (pH)	16 bc	16 bc	21 ab	16 b	18 bc	5	30
G + β-c	28 a	26 a	24 ab	31 a	33 a	42.5	5
pH + β-c	22 ab	22 ab	26 a	18 b	28 ab	22.5	2.5

[a] Mean rating (± standard deviation) of the sensory attributes on scale 0-10

[b] Detected different (more sweet, less sour/bitter/astringent) by the number of panelists out of total 40 panelists. Differences were rated on a category scale 1–5 (1 = notably less attribute; 3 = no difference; 5 = notably more attribute) and transformed to 0/1 data based if differences were found or not. Statistical differences between samples (if detected more often as different compared to the untreated sample) is based on McNemar's test ($p<0.05$) and shown with letters a-c in each.