The impact of bioprocessing on lingonberry flavour was studied by sensory evaluation and chemical analysis (organic acids, mannitol, phenolic compounds, sugars and volatile compounds). Bioprocessing of lingonberries with enzymes, lactic acid bacteria (LAB) or yeast, or their combination (excluding pure LAB fermentation) affected their perceived flavour and chemical composition. Sweetness was associated especially with enzyme treatment but also with enzyme + LAB treatment. Yeast fermentation caused significant changes in volatile aroma compounds and perceived flavour, whereas minor changes were detected in LAB or enzyme-treated berries. Increased concentration of organic acids, ethanol and some phenolic acids correlated with perceived fermented odour/flavour in yeast fermentations, in which increase in benzoic acid level was significant. In enzymatic treatment decreasing anthocyanins correlated well with decreased perceived colour intensity. Enzyme treatment is a potential tool to decrease naturally acidic flavour of lingonberry. Fermentation, especially with yeast, could be an interesting new approach to increase the content of natural preservatives, such as antimicrobial benzoic acid.

1. Introduction

Lingonberry (Vaccinium vitis-idaea) is one of the most important harvested wild berries in Finland. However, only a small amount of berries is picked and used by the food industry (Turtiainen, Salo, & Saastamoinen, 2011). One of the hindrances for wider use of lingonberries is the intense, sour or even bitter flavour. The flavour of lingonberries is typically very acidic and sour due to high amount of organic acids, especially citric acid (Mikulic-Petkovsek, Schmitzer, Slatnar, Stampar, & Vebeic, 2012; Viljakainen, Visti, & Laakso, 2002). The high amount of organic acids leads a pH of below 4. Lingonberries also contain relatively high amount of sugars but the sweetness is masked by acids. Besides organic acids, the concentration of benzoic acid is high in lingonberries (Viljakainen et al., 2002). Benzoic acid is one of the most used chemical preservatives in foods. It has been shown to inhibit both the growth of yeast and bacteria at low pH (Brul & Coote, 1999). The concentration of flavour-active compounds, such as sugars, organic acids, phenolic compounds, volatile compounds, and hence the flavour of lingonberries is also affected by season, maturity and geographic origin.

Neither the sensory quality nor chemical composition of lingonberries has been widely studied. Concerning the chemical composition, most studies have focused on identification of phenolic compounds (e.g., Ek, Kartimo, Mattila, & Tolonen, 2006; Kylli et al., 2011), organic acids and sugars (e.g., Mikulic-Petkovsek et al., 2012; Viljakainen et al., 2002). The composition of volatile compounds has only been studied from the extracted essential oil (Anjou & von Sydow, 1967). In contrast to the chemical composition, the antioxidant activity of lingonberries has received much attention (e.g., Kähkönen, Heinämäki, Ollilainen, & Heinonen, 2003; Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005; Viljanen, Kylli, Kivikari, & Heinonen, 2004). Lingonberries have been shown to inhibit very effectively lipid and LDL oxidation in different model systems. They are also good free radical scavengers.

Bioprocessing with fermentation provides a potential tool to prepare food products with special sensory and chemical properties (Bourdichon et al., 2012). Food fermentations can be carried out spontaneously or by using microbial starter cultures. However lingonberries are a challenging material for fermentation due to acidity and high concentration of antimicrobial phenolic constituents, such as benzoic acid, which is active at low pH values (Viljakainen & Laakso, 2002). As a result attempts to modify the acidic mouthfeel of lingonberry juice using malolactic fermentation with Oenococcus oeni (malic acid converted to less acidic lactic acid) was not successful (Viljakainen & Laakso, 2002).
fermentation of lingonberries usually requires the reduction of the antimicrobial constituents and the addition of sugar (Visti, Viljakainen, & Laakso, 2003).

The aim of the study was to investigate how bioprocessing with different microbes and enzymes affect the sensory characteristics of lingonberries and to correlate the findings with chemical composition of the material. Our approach was not to add any additives to fermentation, but rather use microbial strains, which were able to utilise the berry raw-material as such. In addition to chemical analysis of non-volatile and volatile flavour-active compounds, descriptive sensory analysis was used to gain understanding on how different microbes and enzymes affect perceived flavour.

2. Materials and methods

2.1. Berry material

Frozen, ripe lingonberries (Vaccinium vitis-idaea) of Finnish origin were obtained from a local distributor, Pakkasmarja Ltd. (Suonenjoki, Finland). The lingonberries were frozen and stored at −20 °C until use.

2.2. Bioprocessing of lingonberries

Six types of lingonberry samples were studied: (1) reference material without bioprocessing, (2) yeast fermented material, (3) LAB fermented material, (4) yeast and LAB fermented material, (5) enzyme treated material, and (6) enzyme-treated and LAB fermented material.

For microbial fermentations, frozen lingonberries and ultra-pure water were mixed together (1:1) and heated at 80 °C for 5 min. Subsequently the mixture was cooled in an ice bath and berries were crushed by a sterile potato masher. The pH of the mixture was adjusted to pH 5.0 with 5 N sodium hydroxide. The lingonberry fermentations were started either with Lactobacillus plantarum VTT E-78076 (LAB) or Hanseniaspora uvarum VTT C-11885 (yeast), or with both microbes. Our preliminary experiments indicated that these strains are able to grow in the lingonberry material (data not shown). The microbes were pre-grown in food-grade media. For LAB general edible medium (Saarela et al., 2004) and 24 h incubation at 30 °C in anaerobic conditions was used. Yeast was grown in 0.5 l yeast extract sucrose broth (yeast extract 1.0 g, sucrose solution (10% w/v) and 0.5 l pretreated wort (12%; 6 cfu/g of LAB and/or with approximately 10⁶ cfu/g of LAB and/or with approximately 10⁶ cfu/g of yeast). The fermentations (8 kg each) were carried out in a 15-l capacity bioreactor for 3 days at 30 °C (LAB) or 7 days at 25 °C (other) under constant mixing (130 rpm). LAB fermentations were purged with sterile nitrogen gas to create anaerobic conditions. The viable counts of LAB and yeasts were measured before and after the fermentations using plate count technique as previously described and expressed as colony-forming units (CFU) per gram of wet weight (w.w.) (Katina et al., 2007). The pH value was measured with a standard pH meter. All analyses were performed from triplicate samples.

For the enzymatic treatment frozen lingonberries were thawed in cold-storage room (+4 °C) over night and crushed with a potato masher. The berry mash was heated for 10 min in a mixing reactor (45 °C), after which the enzyme mixture (Biopectinase Super 8×, 100 nkat/g and Acid Protease A, 0.1% w/w) was added. The berry mash was incubated by continuous mixing at 45 °C for 4 h (=enzymatic treatment). For LAB fermentation, the enzyme-treated materials were mixed with water, heat-treated and pH adjusted as for previous fermentations. Enzyme-treated material inhibited the growth of the yeast and was therefore not studied. Bioprocessed lingonberry materials were stored frozen prior to use in sensory evaluation and chemical analyses.

2.3. Sensory analysis

Frozen bioprocessed lingonberry samples were thawed before sensory analysis. Enzyme-treated sample and ultra-pure water were mixed together (1:1) and heated at 80 °C for 5 min. After that the mixture was cooled in an ice bath and the pH of the mixture was adjusted to 5.0 with 5 N sodium hydroxide.

Descriptive sensory analysis (Lawless & Heymann, 2010) was carried out at the sensory laboratory of VTT, which fulfils the requirements of the ISO standards (ISO 1985; ISO 1988). The sensory panel consisted of 11 trained assessors skilled in the sensory assessment of diverse plant-based samples. The vocabulary of the sensory attributes was developed by describing differences between various bioprocessed lingonberry samples. The evaluated attributes of the lingonberry samples were: fresh, lingonberry intensity, sour and fermented odour and flavour, sweetness, bitterness, colour redness and clarity, thickness, amount of berry pieces, and intensity of possible off-taste. The attribute intensities were rated on continuous unstructured, graphical intensity scales. The scales were 10 cm in length and verbally anchored at each end, the left side of the scale corresponding to the lowest intensity (value 0) and the right side to the highest intensity (value 10) of the attribute. The panel members practiced the evaluation of attribute definitions and their intensities during training sessions. The definitions of the descriptors served as a guide for the subjects during testing to minimise confusion over the meaning of each attribute. Panelists had also the possibility to describe verbally the characteristics of the samples.

The berry samples were blind-coded by using 3-digit numbers and presented to the trained assessors in random order from 50-ml disposable plastic containers covered by lids. The lingonberry samples were evaluated crushed. Water was served to the assessors for cleansing the palate between the samples. The samples were judged in two replicate sessions. The scores were recorded and collected using a computerized data system (Compusense Five, Ver 5.4.15, CSA, Computerized Sensory Analysis System; Compusense Inc., Guelph, ON, Canada).

2.4. Analysis of non-volatile flavour-active compounds

The non-volatile flavour-active compounds, such as organic acids, mannitol, phenolic compounds and sugars were analysed by HPLC. Non-volatile chemical compounds were extracted from freeze-dried fermented lingonberries with methanol (100 mg of freeze-dried material/2 mL methanol; HPLC grade, Rathburn Chemicals, Ltd., Walkerburn, UK).

2.4.1. Phenolic compounds

Phenolic compounds were determined by using an analytical HPLC method modified from methods described by Aaby, Ekeberg, and Skrede (2007), and Määttä-Riihinen, Kamal-Eldin, and Törrönen (2004). The HPLC system consisted of a Waters 600S system controller pump, Waters 717 plus autosampler and Waters 2996 series photodiode array detector (Waters Corporation, Milford, MA). Analytes were separated using a Hypersil BDC C-18 (150 × 4.6 mm, 5 μm; Agilent, Santa Clara, CA) reverse-phase column. The data was processed by Waters Empower Pro chromatography software. The solvents used for analyses of lingonberry phenolic compounds in the gradient program were 5% formic acid in water and 100% acetonitrile (Rathburn Chemicals Ltd.). Total analysis time was 45 min. Selected phenolic compounds were
identified and quantified on the basis of corresponding standards (mg/g of dry weight).

### 2.4.2. Glucose, fructose and sucrose

The concentration of sugars (glucose, fructose and sucrose) were analysed by high-performance anion exchange chromatography (HPAEC) (Dionex ICS-3000) with pulse amperometric detection (PAD) (Dionex Corporation, Sunnyvale, CA). The pre- and separation columns (Dionex CarboPac PA-1) were employed at 30 °C with a flow rate of 1 mL/min using the following eluents: milli-Q water, 100 mM NaOH, 300 mM Na acetate/100 mM NaOH and 300 mM NaOH. The eluant gradients were adapted from Tenkanen and Siikala-aho (2000).

### 2.4.3. Organic acids and mannitol

The separation of organic acids and mannitol was carried out on an Aminex HPX-87H column (300 × 7.8 mm; BioRad, Hercules, CA) with a Waters 2690 HPLC separation module and Alliance autosampler, Waters 410 refractive index and Waters 2487 UV detectors, and Waters Empower Pro chromatography software for data processing (Waters Corporation). The solvent used in the isocratic elution was 2.5 mM sulphuric acid. On the basis of the corresponding standard compounds, lactic acid, citric acid, acetic acid and mannitol were quantified (mg/g of dry weight).

### 2.5. Analysis of volatile odour compounds

For analysis of volatile compounds, 2 g of lingonberry samples were weighed into 20-mL headspace vials containing 100 µL of saturated NaCl solution. Toluene was used as internal standard (87 ng/sample). Samples were analysed by SPME–GC/MS according to Viljanen, Lille, Heinio, and Buchert (2011). Samples were pre-incubated at 35 °C for 30 min in closed headspace vials. Extraction of volatile compounds was done at 35 °C for 60 min with a preconditioned (300 °C, 1 h) 75 µm Carboxen/PDMS SPME-fibre (Supelco, Bellefonte, PA). After extraction the analytes were desorbed for 5 min at 260 °C in the splitless injector (split flow 19.4 mL/min) of the gas chromatograph (Agilent 6890 Series, USA) combined with an MS detector (Agilent, 5973Network MSD, USA) and SPME autosampler (Combial; CTC, Zwingen, Switzerland). Analytes were separated on an Ultra-2 capillary column (60 m × 0.25 mm × 1 µm; Agilent Technologies, USA), with a constant flow of 1.5 mL/min, using helium as carrier gas. The temperature programme started at 45 °C with 3 min holding time, then increased 10 °C/min up to 100 °C, followed by 5 °C/min increase up to 150 °C and finally a 10 °C/min increase up to 300 °C, where the temperature was kept for 9 min. MSD was operated in electron-impact mode at 70 eV, in full scan m/z 40–550. The ion source temperature was 230 °C and the interface was 280 °C. Compounds were tentatively identified by comparing their mass spectra with those in the Palisade Complete 600 K Mass Spectral Library (Palisade Corporation, Newfield, NY). Each sample was analysed for four times and normalised peak areas were expressed with respect to internal standard (compound area/ISTD area).

For ethanol measurement, 500 mg of sample were weighed into a 20-mL headspace vial. 1-Butanol was used as internal standard. Ethanol was extracted from the headspace using a Tekmar 7000 autosampler (Teledyne Tekmar, Mason, OH) at 80 °C for 20 min. The autosampler was combined with an Agilent 6890 gas chromatograph and analytes were separated on a Solgel-WAX column (30 m × 0.33 mm; 0.5 µm) (SGE, Ringwood, Australia). Ethanol was detected with a flame ionisation detector at 250 °C. The inlet temperature was 220 °C and split ratio was 0.6:1. The gas chromatograph was operated in constant flow mode with helium as carrier gas at 1.7 mL/min. The oven was programmed from 60 °C to 200 °C with total run time of 12 min. Amount of ethanol (mg/g of sample) in samples was calculated by using ChemStation software (Agilent) with standard curve prepared from AAS grade ethanol (Alko Oy, Helsinki, Finland). Each sample was analysed in triplicate and the mean of these values was used in further calculations.

### 2.6. Statistical data analysis

The chemical composition data were analysed using analysis of variance (ANOVA) and Tukey’s Honestly Significant Difference (HSD) test (significance of differences at p < 0.05) (SPSS 19.0 for Windows, SPSS Inc., Chicago, IL).

Means of the sensory raw data (i.e. scores given by the assessors) obtained from both sensory sessions were calculated. The significance of each descriptive attribute in discriminating between the samples was analysed using ANOVA and Tukey’s HSD test (significance of differences at p < 0.05; SPSS 19.0 for Windows). When the difference in ANOVA among the samples was statistically significant, pairwise comparisons of these samples were performed using Tukey’s test.

The intensities of the perceived attributes of the sensory profile and the non-volatile and volatile chemical compounds of the lingonberry samples were related statistically by PLS (partial least squares) regression using Unscrambler software (Unscrambler 9.8, CAMO Software AS, Oslo, Norway). In the PLS regression only volatile compounds that were found to be significantly different from reference (untreated lingonberries) sample were used. The sensory data used for the multivariate analysis was means calculated over all panellists, and the means of the levels of the chemical compounds were used for the PLS regression. Both the sensory and chemical data were standardised, and the results of the chemical compounds were normalised before the multivariate analysis in Unscrambler. The model was validated by cross-validation. PLS regression is specifically designed to determine relationships existing between blocks of dependent (Y sensory) and independent (X instrumental) variables by seeking underlying factors common to both sets of variables (Martens & Naes, 1998).

### 3. Results and discussion

#### 3.1. Viable cell counts and pH

After the 7-day yeast fermentation (H. uvarum), the viable yeast counts in the lingonberry material were slightly lower than at the beginning (1.1 × 10⁷ ± 1.7 × 10⁴ and 6.7 × 10⁴ ± 4.6 × 10², respectively). During the 3-day fermentation of the lingonberry material with LAB, an approximately 10-fold increase in viable counts of LAB was detected (from 9.9 × 10⁵ ± 1.7 × 10⁴ to 6.7 × 10⁵ ± 4.6 × 10⁴ cfu/g). The lingonberry material that was co-fermented with LAB and yeast contained approximately 2 × 10⁷ CFU/g both LAB and yeast at the end of the 7-day fermentation. In the enzyme-treated lingonberry material the viable counts of LAB showed approximately 10-fold decrease during the 3-day fermentation. Thus, the enzyme-treated lingonberry material appeared to be growth-inhibitory to this strain.

The final pH values in all lingonberry materials varied from 4.6 to 4.9. Hence the changes in pH values during the fermentations were negligible, ranging from 0.05 to 0.1 pH units (data not shown).

#### 3.2. Sensory evaluation of lingonberry

The sensory profile of lingonberry samples showed that processing caused significant changes in all other assessed attributes except in sourness odour (Fig. 1). The enzyme treatment alone...
and together with LAB fermentation decreased the redness of lingonberry, colour clarity and thickness. In another study enzymatic treatment has been shown to influence sensory profile and chemical composition of blackcurrant juice by significantly improving juice yield, but simultaneously increasing fermented and astringent notes (Laaksonen et al., 2012). The largest and most abundant berry pieces were found in reference and LAB-fermented lingonberries. In addition, the freshness (odour/flavour), and lingonberry flavour/odour was most intense in the reference and LAB-fermented lingonberries. As expected, fermented odour and flavour, as well as bitter and sour taste, were most noticeable in yeast and LAB + yeast fermented lingonberries. Bitterness increased after enzymatic treatment but not as much as after fermentation. On the other hand, the sweet taste was least intense in these samples. Moreover, yeast and LAB + yeast fermentation caused most intense off-taste for the samples.

3.3. Non-volatile flavour-active compounds in lingonberries

Selected non-volatile and volatile chemical compounds were analysed with the aim to identify chemical flavour-active compounds responsible for the changes in perceived flavour. The flavour of lingonberries is due to relative amounts and interactions of many different compounds (Ek et al., 2006; Kylli et al., 2011; Lee & Finn, 2012; Mikulic-Petkovsek et al., 2012; Viljakainen et al., 2002). Microbial fermentations consumed simple sugars with concomitant formation of ethanol and/or organic acids. The concentration of both glucose and fructose decreased in samples fermented with yeast and yeast + LAB by 94% (Table 1). This correlated with formation of ethanol (Table 3). It has been previously reported that H. uvarum ferments glucose and fructose to ethanol at the same speed under oxygen limiting conditions (Moreira, Mendes, Guedes de Pinho, Hogg, & Vasconcelos, 2008). Glucose concentration decreased by 45% in enzyme + LAB treated lingonberries. In addition, the concentration of sucrose decreased by more than 96% in samples treated with enzymes and enzymes + LAB, probably due to acid hydrolysis of sucrose at the high temperature used in the enzyme treatment.

The fermentation of lingonberries with LAB and yeast alone as well as together produced small amounts of lactic acid. Possibly contaminating LAB were responsible for lactic acid production in the yeast fermentations although LAB could not be detected (<100 cfu/g) at the end of the 7-day incubation period. In addition, the yeast fermentation with and without LAB fermentation caused most intense off-taste for the samples.

![Fig. 1. Sensory profile of lingonberry samples evaluated by a trained panel (n = 2 x 11).](image)

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Ref</th>
<th>Enzymatic</th>
<th>Yeast</th>
<th>LAB</th>
<th>Yeast + LAB</th>
<th>Enzymatic + LAB</th>
<th>Flavour description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>99.6 ± 7.53 b</td>
<td>140 ± 3.63 a</td>
<td>103 ± 3.45 b</td>
<td>109 ± 7.10 b</td>
<td>105 ± 2.93 b</td>
<td>64.0 ± 6.53 a</td>
<td>Sour</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>nd a</td>
<td>nd a</td>
<td>2.91 ± 2.57 a</td>
<td>18.05 ± 1.79 b</td>
<td>5.86 ± 4.35 a</td>
<td>0.51 ± 0.88 a</td>
<td>Sour, unpleasant</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>nd a</td>
<td>nd a</td>
<td>5.18 ± 3.50 b</td>
<td>nd a</td>
<td>5.86 ± 1.49 b</td>
<td>nd a</td>
<td>Sour</td>
</tr>
<tr>
<td><strong>Sugars and sugar alcohols</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>23.0 ± 0.01 c</td>
<td>0.80 ± 0.00 a</td>
<td>15.0 ± 0.00 b</td>
<td>20.9 ± 0.01 c</td>
<td>12.7 ± 0.01 b</td>
<td>0.80 ± 0.00 a</td>
<td>Sweet</td>
</tr>
<tr>
<td>Glucose</td>
<td>248 ± 0.03 b</td>
<td>253 ± 0.01 a</td>
<td>12.8 ± 0.00 a</td>
<td>246 ± 0.02 b</td>
<td>12.5 ± 0.01 a</td>
<td>235 ± 0.03 b</td>
<td>Sweet</td>
</tr>
<tr>
<td>Fructose</td>
<td>260 ± 0.01 b</td>
<td>267 ± 0.04 a</td>
<td>11.8 ± 0.01 a</td>
<td>258 ± 0.01 b</td>
<td>10.4 ± 0.01 a</td>
<td>241 ± 0.02 b</td>
<td>Sweet</td>
</tr>
<tr>
<td>Mannitol</td>
<td>nd a</td>
<td>nd a</td>
<td>171 ± 15.7 b</td>
<td>nd a</td>
<td>170 ± 4.39 b</td>
<td>nd a</td>
<td>Sweet</td>
</tr>
<tr>
<td><strong>Phenolic compounds</strong></td>
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<tr>
<td>p-Coumaric acid</td>
<td>0.13 ± 0.00 a</td>
<td>0.02 ± 0.00 a</td>
<td>0.37 ± 0.00 b</td>
<td>0.11 ± 0.00 a</td>
<td>0.43 ± 0.00 b</td>
<td>0.10 ± 0.00 a</td>
<td>Balsamic, bitter</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.01 ± 0.00 a</td>
<td>0.03 ± 0.00 a</td>
<td>0.10 ± 0.00 a</td>
<td>0.01 ± 0.00 a</td>
<td>0.29 ± 0.00 b</td>
<td>0.03 ± 0.00 a</td>
<td>Bitter</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>0.71 ± 0.00 a</td>
<td>0.96 ± 0.00 a</td>
<td>4.41 ± 0.00 a</td>
<td>0.88 ± 0.00 a</td>
<td>6.47 ± 0.01 a</td>
<td>0.87 ± 0.00 a</td>
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</tr>
<tr>
<td>Benzoic acid</td>
<td>6.69 ± 0.01 a</td>
<td>10.1 ± 0.00 a</td>
<td>14.2 ± 0.01 a</td>
<td>7.24 ± 0.02 a</td>
<td>15.1 ± 0.02 a</td>
<td>9.37 ± 0.02 b</td>
<td>Balsam, urine, sour, pungent</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.19 ± 0.01 a</td>
<td>3.67 ± 0.00 a</td>
<td>0.87 ± 0.00 a</td>
<td>0.42 ± 0.00 a</td>
<td>4.02 ± 0.00 a</td>
<td>3.25 ± 0.01 b</td>
<td>Bitter</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>4.34 ± 0.01 a</td>
<td>1.91 ± 0.00 a</td>
<td>4.09 ± 0.01 a</td>
<td>3.26 ± 0.01 a</td>
<td>3.65 ± 0.01 a</td>
<td>0.77 ± 0.00 a</td>
<td>Odourless, flavourless, astringent</td>
</tr>
</tbody>
</table>

**nd** not detected.

*Values in the same row followed by different letters are significantly different (p < 0.05).

A Flavour descriptions adapted from Acree and Arn, 2010.
produced some acetic acid. However, the level of these two organic acids remained low, under 20 mg/g dry matter. Acetic acid is produced during yeast fermentation from enzymatic oxidation of acetate (Díaz-Montaño & de Jesús Ramírez Córdova, 2009). A slightly increase was also seen in enzymatically treated samples.

Selected phenolic compounds, benzoic acid, p-coumaric acid, caffeic acid, quercetin and anthocyanins were quantitated by HPLC (Table 1). The distribution of these selected phenolic compounds is in accordance with the literature (Mattila, Hellström, & Törönen, 2006). The concentration of benzoic acid increased during fermentation in almost all lingonberry samples compared to reference lingonberry sample and other fermentations. Quercetin and kaempferol, which are the main flavonols in lingonberry, exist mainly as various sugar conjugates in the berry fruit, in which the amount of free quercetin is endogenous and microbially produced glycosidases can release the more volatile compounds and have a major impact on sensory properties (Claus, 2009).

Selected phenolic compounds, benzoic acid, p-coumaric acid, caffeic acid, quercetin and anthocyanins were quantitated by HPLC (Table 1). The distribution of these selected phenolic compounds is in accordance with the literature (Mattila, Hellström, & Törönen, 2006). The concentration of benzoic acid increased during fermentation in almost all lingonberry samples compared to reference lingonberry sample without any fermentation. Benzoic acid levels were highest in lingonberry samples fermented with yeast and yeast + LAB. In addition, the concentration of p-hydroxybenzoic acid increased in yeast and yeast + LAB fermented samples, compared with reference lingonberry sample and other fermentations. Slight increase was also seen in enzymatically treated samples. The concentration of both p-coumaric acid and caffeic acid was relatively low in all samples (under 0.4 mg/g dry matter). The highest concentrations of these phenolic acids were found in samples fermented with yeast and yeast + LAB. It is known that many aromatic compounds, such as terpenes, anthocyanins and phenolics are stored in wine grapes in a glycosidically-bound form. Added, endogenous and microbially produced glycosidases can release the more volatile compounds and have a major impact on sensory properties (Claus, 2009).

Highest quercetin concentrations were found in yeast + LAB fermented lingonberries as well as enzyme treated lingonberries with and without LAB fermentation. Quercetin and kaempferol, which are the main flavonols in lingonberry, exist mainly as various sugar conjugates in the berry fruit, in which the amount of free quercetin...
The concentration of total anthocyanins remained unchanged in lingonberry samples fermented with yeast and LAB alone or together. On the contrary, the anthocyanin concentration decreased in enzymatically treated lingonberry samples with and without LAB fermentation. The stability and the profile of anthocyanins are greatly affected by the glycosidase side activities present in the enzyme preparations, which are able to hydrolyse certain anthocyanin aglycones. The aglycones are greatly affected by the glycosidase side activities present in LAB fermentation. The stability and the profile of anthocyanins in enzymatically treated lingonberry samples with and without LAB fermentations were very gentle to phenolic compounds; the concentration remained unchanged for all of those compounds. The concentration of total anthocyanins remained unchanged in lingonberry samples fermented with yeast and LAB alone or together. On the contrary, the anthocyanin concentration decreased in enzymatically treated lingonberry samples with and without LAB fermentation. The stability and the profile of anthocyanins are greatly affected by the glycosidase side activities present in the enzyme preparations, which are able to hydrolyse certain anthocyanins to the corresponding aglycones. The aglycones are not affected by this enzyme preparation. Purified enzymes or enzyme mixtures with known activity profiles could offer an interesting tool to modify berry material and also tailor sensory properties not affected by this enzyme preparation.

Fermentation alone was very gentle to phenolic compounds; the concentration remained unchanged for all of those compounds. However, these changes are mostly caused by the fermentation conditions, not by the LAB (data not shown).

### 3.4. Volatile flavour-active compounds in lingonberries

From lingonberry samples 38 volatile chemical compounds were identified by SPME–GC/MS (Table 2); 8 aldehydes, 6 ketones, 7 alcohols, 7 terpenes, 5 esters, 2 acids and 3 other compounds. A
The chromatogram of untreated lingonberries is presented in Fig. 4. In the literature, only one research paper was found on volatile compounds in lingonberries (Anjou & Von Sydow, 1967), in which essential oil was extracted from berries, which differs from the SPME–GC/MS analysis used in this study.

The amount and composition of volatile chemical compounds changed during fermentations and enzymatic treatments. The amount of diacetyl, ethyl acetate, ethyl propionate, acetoin, diethyl acetal, 3-methyl-1-butanol, 2-methyl-1-butanol, isoamyl acetate, 6-methyl-5-hepten-2-one, benzyl alcohol, acetophenone, phenylethyl alcohol, ethyl benzoate and β-selinene increased in samples fermented with yeast and yeast + LAB. In particular, the levels of all measured esters increased in lingonberries fermented with yeast or with yeast + LAB. The level of ethyl acetate in yeast and yeast + LAB fermented lingonberries is more than 1000 times higher than in reference lingonberries.

Diacetyl is small ketone that has butter flavour. It is produced normally in both LAB and yeast fermentation (Romano & Suzzi, 1996; Suomalainen & Ronkainen, 1968; Walsh & Cogan, 1973). In addition, acetoin, which also has butter and cream odour, is produced in the same route as diacetyl so it is logical that they are both increased in yeast and yeast + LAB fermentation. Diacetyl was found in yeast and yeast + LAB fermented lingonberries at more than 7-fold higher levels than in reference untreated lingonberries. Phenylethyl alcohol is produced by reduction of acetophenone (Rogers, Hackman, Mercer, & DeLancey, 1999), and these two compounds are commonly found in yeast fermented foods and beverages such as beer and wine. Acetophenone was found in yeast and yeast + LAB fermented lingonberries around two times higher than in reference lingonberries and it has flower and almond odour. Phenylethyl alcohol has honey, spice, rose and lilac odour and was found only in yeast and yeast + LAB fermented lingonberries.

Fig. 2. Relation of sensory perception and non-volatile compounds of processed lingonberry. The samples are marked in green (reference, yeast ferm yeast fermentation, enzyme = enzymatic treatment, LAB ferm = LAB fermentation, yeast & LAB = yeast and LAB fermentation, enzyme & LAB = enzymatic treatment and LAB fermentation), chemical compounds in blue and sensory characteristics in red (prefix O = odour, F = flavour, Col = colour).

Fig. 3. Relation of sensory perception and volatile compounds of processed lingonberry by PLS regression. The samples are marked in green, chemical compounds in blue and sensory attributes in red. Abbreviations are as stated in the text of Fig. 2.
The level of most aldehydes remained unchanged or decreased during yeast and LAB fermentations and enzyme treatments. The decreased levels of these aldehydes may be due to their degradation and oxidation. The decreased levels of aldehydes is probably also due to the fact that the acidic pH (4.6–4.9), used in these treatments is not optimal for endogenous enzymes, such as lipoxygenase and hydroperoxide lyase. Lipoxygenase has been shown to oxidise endogenous fatty acids, and hydroperoxide lyase decomposes formed fatty acid hydroperoxides to different aldehydes and ketones. If the activities of these enzymes are inhibited, degradation is more favoured than formation. The level of 6-methyl-5-hepten-2-one increased in yeast fermented lingonberries. 6-Methyl-5-hepten-2-one is a degradation product of lycopene. Lingonberries contain some carotenoids, but not in high quantities.

LAB fermentation alone and together with enzymatic treatment increased most the level of 2-pentanone. The level of benzyl alcohol increased in all samples compared to the reference sample. It seems that enzymatic treatment alone or in combination with LAB fermentation has little effect on terpenes, but in some cases terpene levels were increasing. α-Pinene levels significantly increased with enzymatic treatment whereas with other bioprocesses α-pinene levels decreased or remained unchanged. Lingonberries fermented with yeast or with yeast + LAB had the highest levels of β-selinene compared to reference lingonberries or lingonberries with other fermentation and enzymatic treatments. According to these results enzymatic treatment in combination with LAB fermentation decreased most the levels of terpenes.

3.5. Comparison of sensory and chemical analysis

The chemical composition of non-volatile compounds of bio-processed lingonberries was related to sensory perception (Fig. 2). Logically anthocyanins are related to redness, glucose and fructose to sweetness, and ethanol, phenolic and some organic acids to sour taste, bitterness and fermented odour/flavour. On the other hand, lactic acid is responsible for sour odour. Volatile compounds were also related to sensory perception as seen in Fig. 3. Statistical multivariate analysis (PLS regression) showed excellent correlation between the sensory and chemical results: The two first principal components explained 88% of the variation in chemical and 90% in sensory results. Yeast fermentation alone or together with LAB fermentation resulted in fermented odour and flavour, sour, bitter taste and off-taste, which were related to phenolic acids (p-coumaric, caffeic and benzoic acids), citric and acetic acid, mannitol, ethanol and pH, and diacetyl and several other volatile compounds. Reference and LAB-fermented lingonberries were fresh and lingonberry-like in flavour, and were related to octanal and nonanal. Both nonanal and octanal have as pure compounds lemon, citrus, green flavour. Enzyme treatment alone and together with LAB fermentation was related to 3-methylbutanoic acid which has sweet, rancid and acidic flavour. Most changes in volatile composition are related to yeast and yeast & LAB fermentations; for example the amount of diacetyl, ethyl benzoate, ethyl acetate, ethyl propionate, 3-methyl-1-butanol, 2-methyl-1-butanol, phenylethyl alcohol, diethyl acetal, 2-hydroxy-2-butanone, and (E)-2-butenal.

Enzyme and enzyme-LAB treated lingonberries were linked to sweetness, and glucose and fructose, as well as 3-methylbutanoic acid. LAB fermentation of the lingonberry material was poor and did not seem to have impact on the sensory profile of lingonberry. Redness duly correlated with anthocyanins. Thus, yeast fermentation was shown to cause a non-desired fermented odour and flavour to lingonberry, whereas enzyme treatment was shown to increase the sweetness of lingonberry. Excessive formation of ethanol could be controlled by oxygenation as H. uvarum produces ethanol only under oxygen limitation (Díaz-Montaño & de Jesús Ramírez Córdova, 2009).

4. Conclusions

Fermentation and enzymatic treatment are possible bioprocessing pathways to modify lingonberry flavour, and thus they could enhance the use of lingonberries in food and beverage product formulation. Enzyme treatment was the most potent processing method to modify lingonberry taste, as it increased sweetness of this naturally very acidic berry. Yeast fermentation resulted in fermented odour and off-taste. However, optimisation of the fermentation process could be used to improve the flavour of the product, e.g., by reducing the amount of ethanol. Fermented berries, on the other hand, contained higher levels of phenolic compounds, which could give extra health advantages to the products, and also as natural preservatives, could increase preservation of food and beverage products.

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