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# Comparison of volatile compounds and sensory profiles of alcoholic black currant (*Ribes nigrum*) beverages produced with *Saccharomyces*, *Torulaspora*, and *Metschnikowia* yeasts

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ABSTRACT

Black currants (*Ribes nigrum*) were fermented with *Saccharomyces* and non-*Saccharomyces* yeasts without added sugar to yield low-ethanol-content beverages. The effects of yeasts on the volatile compounds and sensory characteristics were analysed by HS-SPME-GC-MS, GC-O, and generic descriptive analysis. Ninety-eight volatile compounds were identified from the black currant juice and fermented beverages. Significant increases in the contents of esters (131 %), higher alcohols (391 %), and fatty acids (not present in juice sample) compared to initial juice were observed depending on the yeasts used. GC-O analysis revealed the higher impact of esters on the sensory properties of *Saccharomyces bayanus*-fermented beverage compared to the *Torulaspora delbrueckii*-fermented beverage. In the sensory evaluation, non-*Saccharomyces* yeasts resulted in a higher 'black currant odour'. However, all beverages were intensely sour, which can be a significant challenge in the development of alcoholic berry beverages.

## 1. Introduction

Black currant (*Ribes nigrum*) is a widely cultivated berry crop in Finland and Europe. Black currants have a typical attractive odour, and the volatile composition of black currants has been widely studied. The main volatile compounds in black currants are esters, terpenoids, and alcohols, of which esters and terpenoids have the major contribution to the overall aroma. (Jung et al., 2017; Liu et al., 2018; Marsol-Vall et al., 2018) Additionally, certain thiol and pyrazine compounds, such as 4-methoxy-2-methyl-2-butanethiol, 2-isopropyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine, have been reported as important contributors to the black currant aroma (Jung et al., 2017). Several factors, such as the black currant cultivar, geographical origin (e.g. growth latitude), and processing parameters (e.g. enzymatic treatment and storage temperature), affect the preservability of the black currant volatile profile and content (Marsol-Vall et al., 2018, 2019). The pectin content of the black currant is relatively high, 19 g/kg of berries (Varo et al., 1984), making use of pectinolytic enzymes needed in the black currant juicing process. However, non-enzymatically treated black currant juice has recently been reported to have a more berry-like odour than enzymatically treated black currant juice. In addition, pectinase treatment of black currant reduces the levels of most esters, aldehydes and terpenes (Marsol-Vall et al., 2019). Despite the desirable odour characteristics, black currant taste is very sour due to the high acid contents, resulting in limited exploitation of the berry (Laaksonen et al., 2016).

In fermented fruit products, the selection of wine yeast species and strains affects the production rates of volatile compounds, such as short-chain fatty acids, esters and acetates, higher alcohols, and carbonyl compounds, during alcohol fermentation (Lambrechts & Pretorius, 2000). *Saccharomyces cerevisiae* and *S. bayanus* are the most frequently utilised wine yeasts due to their high ethanol tolerance and good fermentation performance at high sugar content and low pH. *S. bayanus* is especially used to ferment wine at lower temperatures. It also contributes to flavour properties by increasing the contents of esters and acetates, and alternately decreasing the contents of volatile acids (Muñoz-Bernal et al., 2016). The use of non-*Saccharomyces* wine yeasts has gained increasing popularity in recent years, including in non-grape fermentations. They are used to produce alcoholic beverages with reduced ethanol content compared to traditional products. Furthermore, their application may lead to an improvement of the sensory quality by increased formation or release of aroma active compounds from the

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fermented fruits (Escribano-Viana et al., 2018; Yang et al., 2015; Varela, 2016). However, due to their low fermentation performance and low ethanol tolerance, non-*Saccharomyces* yeasts have mainly been used in mixed (simultaneous or sequential) fermentations with *S. cerevisiae*.

*Torulaspora delbrueckii* is the most frequently utilised non-*Saccharomyces* wine yeast. It is the most similar to *S. cerevisiae*, with good fermentation performance, sugar utilisation, and regulation patterns of metabolism (Comitini et al., 2011; Ramírez & Velázquez, 2018). In traditional high-sugar grape must fermentation, *T. delbrueckii* is typically used in combination with *S. cerevisiae* due to its low ethanol tolerance (Bely et al., 2008). In addition, *T. delbrueckii* shows slower growth and fermentation vigour than *S. cerevisiae* during anaerobic fermentation leading to the dominance of *S. cerevisiae* in simultaneous or sequential fermentations. Mixed fermentation with *T. delbrueckii* may improve the comprehensive quality of wine, resulting in a more complex aroma profile, lower production of acetic acid and other undesired volatiles, and lower ethanol content in some cases (Azzolini et al., 2015; Canonico et al., 2019). When used alone, products fermented with *T. delbrueckii* showed low undesirable volatile acidity, glycerol, and ethanol production (Bely et al., 2008).

*Metschnikowia* yeasts are ubiquitous and can be found from spontaneous wine fermentations and the surfaces of many fruits. They usually take part at the beginning of fermentation, and their growth decreases simultaneously with increasing ethanol concentration. Their low ethanol tolerance and low fermentation performance result in the necessary use of *S. cerevisiae* yeast together with *Metschnikowia* in simultaneous or sequential fermentation. Fermentation with *Metschnikowia* yeasts is associated with a more complex aroma profile (Duarte et al., 2019; Suárez-Lepe & Morata, 2012). *M. pulcherrima* has characteristic $\beta$-glucosidase (Fernández et al., 2000) and $\alpha$-L-rhamnosidase (Comitini et al., 2011) activity. Glycosidases cleave volatiles from aroma precursor glycosides, contributing to the complexity of wine aroma (Suárez-Lepe & Morata, 2012). In addition, *M. pulcherrima* and *M. fructicola* are reported to have polygalacturonase activity (Belda et al., 2016), which makes them suitable for fermentation of the juices with high pectin contents, such as black currant juice. *M. fructicola* is reported to have an effect on the wine volatile composition, such as increased formation of ester and acetate, when used in fermentation with *S. cerevisiae* (Boscaino et al., 2019). In addition, *M. pulcherrima* and *M. fructicola* strains have killer yeast activities (Kurtzman & Droby, 2001).

Studies focusing on the effects of yeast fermentation on the volatile composition of black currants are scarce (Leino & Kallio, 1993), although alcoholic black currant wine products have been commercially available for decades. To the best of our knowledge, this is the first time that the effects of fermentation with non-*Saccharomyces* yeasts on the volatile profile and sensory properties of black currant have been studied. In the present study, we aimed to assess the effects of fermentations with non-*Saccharomyces* yeasts on the composition of the volatile compounds and sensory properties of pasteurised black currant juice without using pectinolytic enzymes or additional sugars. Special focuses were *S. cerevisiae*, *S. bayanus*, and *T. delbrueckii* yeasts used in pure inoculations, as well as in sequential fermentations of *M. pulcherrima* and *M. fructicola* with *S. cerevisiae*. Volatile composition was studied using HS-SPME-GC-MS, and potential contributions of the compounds to the odour and flavour quality were investigated with GC-O and descriptive sensory analysis. Yeast fermentation was expected to notably affect the volatile composition and, thus, sensory properties of black currant. In addition, fermentations with the different yeast strains and species were expected to notably affect these properties differently.

## 2. Materials and methods

### 2.1. Materials

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#### 2.1.1. Oenological yeasts

Two *Saccharomyces cerevisiae* yeast products (**SC1** and **SC2**, respectively, W15 and ICV-K1, Lalvin®, Montreal, QC, Canada), *Torulaspora delbrueckii* (**TD**; Biodiva, Level™, Edwardstown, Australia), *Metschnikowia pulcherrima* (**MP;** Flavia, Level™, Edwardstown, Australia), and *M. fructicola* (**MF;** IOC Gaïa, Edwardstown, Australia) were kindly provided by Lallemand Inc. (Montreal, QC, Canada), and *S. bayanus* (**SB;** Condessa, Viinitalo Melkko Ltd, Lahti, Finland) was purchased from the local wine equipment store. All utilised yeasts were active dried yeasts.

#### 2.1.2. Standard compounds

1,8-Cineol, 1-nonanol, 1-octen-3-one, 1-pentanol, 2,3-butanedione, 2-furamethanol, 2-isobutyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, 2-methyl-1-propyl acetate, 2-methylbutanal, (+)-2-methyl butanoic acid, 3-methylbutanal, 3-methyl butanoic acid, 3-methylbutyl acetate, 3-(methylthio)-propional, 4-methoxy-2-methyl-2-butanethiol, 6,10-dimethyl-5,9-undecadien-2-one, 6-methyl-5-hepten-2-one, benzaldehyde, (−)-bornyl acetate, $\beta$-damascenone, ethyl decanoate, ethyl hexanoate, ethyl octanoate, heptanal, heptanol, hexanal, hexanoic acid, hexanol, 3,7-dimethylocta-1,6-dien-3-ol (linalool), methyl butanoate, methyl hexanoate, nonanal, octanal, octanoic acid, 1-isopropyl-4-methylbenzene (*p*-cymene), pentanol, tetrahydro-2-(2-methyl-1-propenyl)-4-methyl-pyran (+/−-rose oxide), *p*-mentha-1,4(8)-diene (terpinolene), (*E*)-2-octenal, an alkane mixture (C5-C20), L-(+)-tartaric acid, xylitol, quinic acid, and galacturonic acid were purchased from Sigma-Aldrich (Saint Louis, MO, USA). D-(−)-fructose and D-(+)-glucose were purchased from Merck (Darmstadt, Germany). 2-Pentanone, methyl 3-methylbutanoate, 4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene ($\beta$-caryophyllene), butanoic acid, *p*-mentha-1,4-diene ($\gamma$-terpinene), *p*-mentha-1,8-diene (limonene), and phenethyl alcohol were purchased from Fluka Chemicals (Neu Ulm, Switzerland). *n*-Butanol was purchased from Riedel-de Haën (Morris Plains, NJ, USA). Ethanol ($\geq$99.5 %) was purchased from ALTIA oy (Rajamäki, Finland). All standards used in GC–MS analysis were a purity of $\geq$ 95 %.

### 2.2. Methods

#### 2.2.1. Preparation of black currant juice and fermented beverages

Frozen black currants (Pakkasmarja, Ltd., Suonenjoki, Finland) were purchased from a local supermarket. Black currant juice (BCJ) was pressed with a horizontal juice press attachment of a food processor (Kenwood Limited, Havant, United Kingdom) and divided into 50 mL glass tubes. Approximately 500 mL juice was obtained from 1 kg of berries. BCJ was immediately pasteurised by immersing the glass tubes in boiling water. The tubes were monitored with a thermometer (TM-947SD, Lutron Electronic Enterprice Co., Ltd., Taipei, Taiwan) until their temperature reached 97 °C, kept at 97 °C for 30 s, and then immediately transferred to ice to cool to 25 °C. For each fermentation, 500 mL of pasteurised juice was measured and transferred to a glass bottle.

Inoculations were performed as described previously by Kelanne et al. (2020). Briefly, all of the yeasts were reactivated with water rehydrant solution (35 g/L, Go-Ferm, Lallemand, Inc., Montreal, Quebec, Canada) for a shorter lag phase at the beginning of fermentation. The following optimal temperatures were used to reactivate each yeast: *S. cerevisiae* 1, 35–40 °C; *S. cerevisiae* 2, 40 °C; *S. bayanus*, 30–35 °C; *T. delbrueckii,* 30 °C; *M. pulcherrima*, 30 °C; and *M. fructicola*, 20–30 °C. The reactivation time for every yeast was greater than 20 min but less than 45 min. The inoculation amount was 0.25 g/L of active dried yeast, corresponding to $1.4 \times 10^8$ colony-forming units (CFU)/mL of *S. cerevisiae* 1, $3.6 \times 10^9$ CFU/mL of *S. cerevisiae* 2, $9.7 \times 10^7$ CFU/mL of *S. bayanus*, $4.7 \times 10^8$ CFU/mL of *T. delbrueckii*, $7.3 \times 10^8$ CFU/mL of *M. pulcherrima*, and $1.2 \times 10^8$ CFU/mL of *M. fructicola*. In the sequential fermentation with *Metschnikowia* yeast and *S. cerevisiae, Metschnikowia* yeasts were inoculated first, and after 24 h, *S. cerevisiae* was inoculated as described above.

The fermentations were monitored at the beginning by weighing to ensure that fermentation had started. Fermentations, expect *S. bayanus* fermentation, were stopped (yeast killer, potassium sulfate/potassium sorbate, 1:1, Jässtopp D, Viinitialo Melkko, Lahti, Finland) after seven days. *S. bayanus* fermentation was stopped after 12 days. The percent soluble solids in an equivalent solution (°Brix) were measured before and after fermentations. All fermentations were prepared in triplicate, and pooled samples were used for sensory evaluations. Samples were stored at −20 °C before analysis or sensory evaluations.

### 2.2.2. Qualitative and quantitative analysis of sugars and organic acids with GC-FID

The sugars and organic acids were analysed by a gas chromatographer (GC, GC-2010Plus, Shimadzu Corp., Kyoto, Japan) equipped with a flame ionisation detector (FID) as trimethylsilyl (TMS; Tri-Sil reagent, hexamethyldisilazane:trimethylchlorosilane:pyridine, 2:1:10, Thermo Scientific, Pierce Biotechnology, Rockford, IL, USA) derivatives as explained previously with slight modifications (Kelanne et al., 2019). External standards (succinic acid, citric acid, fructose, quinic acid, glucose, galacturonic acid, and sucrose, all 5 g/L) were used for identification and quantification of the main sugars and organic acids before and after fermentations. Xylitol and tartaric acid (both 5 g/L) were used as internal standards for quantification of sugars and organic acids, respectively. An aliquot portion of 250 μL of each sample and both internal standards were diluted to 5 mL and filtered with a regenerated cellulose syringe filter (0.45 μm). An aliquot portion of 300 μL of the filtrate was pipetted to an autosampler bottle and evaporated to dryness at 50 °C under nitrogen flow. The samples were stored at a desiccator until analysis but at least overnight. For TMS derivatisation, 500 μL of Tri-Sil reagent was added to dry samples, mixed vigorously for 5 min, and incubated for 30 min at 60 °C. Separation of derived compounds was carried out with a Supelco Simplicity-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm df, Supelco, Bellefonte, PA, USA). The oven was temperature-programmed from 150 °C (hold = 2 min) to 210 °C with a constant ramp of 3 °C/min, and then to 275 °C (hold = 5 min) with a constant ramp of 40 °C/min. The injector temperature was set to 210 °C, and split injection was applied with a split ratio of 1:15. Helium was used as a carrier gas with a linear velocity of 44.8 cm/s (constant flow). The temperature of the FID was set to 290 °C. All samples were analysed in triplicate.

### 2.2.3. Determination of volatile compounds with GC–MS

Volatile compounds in the black currant juice (BCJ) and fermented beverages (FBs) were determined with a GC-2010 gas chromatographer coupled with a GCMS-QP 2010 Plus mass spectrometer (Shimadzu Europa GmbH, St Petersburg, Russia) and equipped with a CTC Combi PAL autosampler (CTC Analytics, Switzerland), an automated headspace solid-phase microextraction (HS-SPME) system. An aliquot portion of 100 μL of each sample and the internal standard (2-octanol at a concentration of 5 mg/L) were transferred into a 20 mL headspace vial. DVB/Carboxen/PDMS (50/30 μm, 2 cm; Supelco, Bellefonte, PA, USA) stable flex SPME fibre was used to extract volatile compounds. Prior to volatile extraction, the samples were equilibrated and stirred thoroughly using a glass-coated magnetic stirrer at 40 °C for 5 min. The SPME fibre was exposed to the headspace of the sample vial for 20 min at 40 °C. Volatiles were thermally desorbed in the injection port at 270 °C. After injection, the fibre was left in the injection port for 20 min for reconditioning. The same volatile enrichment procedure was used with both column types. The column oven was cooled with liquid nitrogen to −10 °C prior to analysis with a medium polar column (Rxi-5 ms, 30 m × 0.25 mm, 1.0 μm, Restek Co., Bellefonte, PA, USA). The oven was temperature-programmed from −10 °C (hold = 1 min) to 280 °C with a constant ramp of 8 °C/min (hold = 1 min). The injector temperature was set to 270 °C, and splitless injection was applied. Helium was used as a carrier gas with a linear velocity of 35 cm/s (constant flow). Mass selective detection was performed in the scan mode (35–350 *m/z*; EI (70 eV)). The interface temperature was set to 280 °C, and the ion source was set to 200 °C.

Analysis of the high polarity analytical column was performed on a ZB Wax -column (20 m × 0.18 mm × 0.18 μm, Phenomenex Inc., USA). The oven was temperature-programmed from 40 °C (hold = 1 min) to 240 °C with a constant ramp of 8 °C/min (hold = 3 min). The injector temperature was set to 250 °C, and splitless injection was applied. Helium was used as a carrier gas with a linear velocity of 35 cm/s (constant flow). Mass selective detection was performed in the scan mode (46–250 *m/z*; EI (70 eV). The interface temperature was set to 220 °C, and the ion source temperature was set to 200 °C.

Identification of the volatile compounds was performed by probability-based matching of the obtained mass spectra with the mass spectra from the National Institute of Standards and Technology database (NIST14) and Adams Essential Oil mass spectral library 2007, as well as from data obtained from the literature (Jung et al., 2017; Leino & Kallio, 1993; Y. Liu et al., 2018; Marsol-Vall et al., 2018, 2019; Varming et al., 2004). As a second criterion for the identification, linear temperature-programmed retention indices (RIs) were calculated for both columns used. Measured RIs were compared to data obtained from authentic reference compounds and from the literature and retention index databases. As it was not the aim to fully quantify the volatile compounds, but rather to compare the impact of the fermentation strategy on the formation of the volatiles, semi-quantitation was performed according to Elmore (2015). Calculation of the relative concentrations of the volatile compounds was performed with the peak areas obtained from analyses on the nonpolar column. Relative concentrations were calculated by dividing the peak area by that of the internal standard (2-octanol, 50 ng/100 μL) area considering a response factor of 1 for each compound. Samples were analysed in quadruplicate.

### 2.2.4. GC-O analysis of black currant juice and fermented beverages

GC-O analysis was performed with a Hewlett-Packard HP6890 Series GC system (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with a flame ion detector (FID) and an olfactometry port (ODP-1, Gerstel GmbH & Co. KG, Mülheim an der Ruhr, Germany). A 9 mL portion of a sample was placed in a 50 mL Erlenmeyer flask. The sample was equilibrated and stirred thoroughly using a magnetic stirrer at 40 °C for 10 min. The SPME fibre was exposed to the headspace of the sample vial for 20 min at 40 °C. A medium polar capillary column (HP-5MS, 30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc., Santa Clara, CA, USA) was used to separate the compounds. The oven was temperature-programmed from 30 °C to 260 °C with a constant ramp of 8 °C/min. The injector temperature was set to 270 °C, and splitless injection was used. Helium was used as a carrier gas with a linear velocity of 35 cm $s^{-1}$ (constant flow). The temperature of the FID was set to 270 °C and the sampling rate was 20 Hz.

Odour frequency, length, and description were collected from the GC-O analysis. Five experienced assessors were recruited in-house. They were all familiarised with the GC-O system and instructed to press the signal button, when the odour impression started, and keep pressing until the end of the odour. The assessors performed sniffing of all samples in duplicate. The nasal impact frequencies (NIFs) were summed from the individual signals (NIF 100 % corresponding to all assessors detecting an odour at the same time) and the square of NIF (SNIF) was calculated by the duration (s) of NIF (≥40 %) × (NIF %/100). Identification of compounds was performed using retention indices and standard compounds and was compared to the results from GC–MS analyses.

### 2.2.5. Sensory evaluation of fermented black currant beverages

The descriptive analysis was performed with 11 assessors (2 men, 9 women, age 20–59). In the first training session, assessors were subjected to a basic test of their ability to recognise tastes from ASTM standardised test solution concentrations.

The training for the generic descriptive analysis consisted of four one-hour sessions (Lawless & Heymann, 2010). In the first session,

assessors were presented with three FB samples and asked to describe their appearance, odour, taste, flavour, and texture followed by a discussion on the given attributes. In further sessions, suitable sensory attributes, reference samples and intensities were agreed upon. The final profile had 12 attributes (Supplementary Table 1) rated on line scales (0–10) using anchored reference samples. All FB samples were presented to assessors at least once during the training sessions. Sensory attributes were evaluated in the same order as described in Supplementary Table 1.

All samples were evaluated in triplicate during three sessions. Ten millilitres of each sample was served monadically in a tulip-shaped standard wine glass covered with a glass lid. The sample presentation order was randomised both among assessors and between sessions. One-minute breaks were set between the samples, and the assessors were instructed to clean their palate by drinking water and chewing a piece of cracker. Sensory evaluation took place in a sensory laboratory (ISO 8589, University of Turku, Finland). Data were collected with Compusense Cloud version 20.0 (West Guelph, Ontario, Canada).

The performance of the panel was monitored with PanelCheck V1.4.2 software (Nofima, Tromsø, Norway) using three-way ANOVA (F values), Tucker-1 plots, and p-MSE plots. None of the assessors showed systematically poor performance in all samples. The exclusion of extreme assessors (one or two) had little or no effect on the statistically significant differences. All assessors were included in the data analysis.

#### 2.2.6. Statistical analysis

SPSS 25.0.0.1. (IBM SPSS Statistics Inc., Chicago, IL, USA) was used to calculate one-way ANOVA with Tukey's test to determine possible significant differences in concentrations of volatile compounds between the samples, and the independent sample *t*-test was used to determine significant differences between the black currant juice and averaged fermented beverage. One-way ANOVA with the LSD test was used to analyse the statistical significance of sensory attributes between samples. Unscrambler X (version 10.4, CAMO Inc, Oslo, Norway) was used to construct the multivariate analyses (PCA and PLS regression).

## 3. Results and discussion

### 3.1. Fermentation kinetics

The success of the fermentations was monitored by measuring the percent soluble solids in an equivalent solution (°Brix) before and after fermentations. The °Brix of black currant juice was 15.8, whereas the °Brix in the fermented beverages was between 8.7 and 8.9. These results were in consensus with the °Brix results reported in Kelanne et al. (2020).

### 3.2. Residual sugars and organic acids in fermented beverages

Pasteurised black currant juice contained sugars at a total content of 59.1 g/L (19.3 g/L fructose, 37.2 g/L glucose, and 2.5 g/L sucrose), citric acid at 21.1 g/L, and quinic acid at 0.5 g/L. After fermentations, the content of residual sugars ranged between 0.19 g/L (SB) and 3.34 g/L (MFSC1). Citric acid was the main organic acid in the black currants, and its content did not change during fermentation (Supplementary 2). Succinic acid and galacturonic acids are formed by yeast or the enzyme activity of yeast during fermentation. Most succinic acid is formed from pyruvate in the reductive branch of the Krebs cycle (Waterhouse et al., 2016). Succinic acid levels were significantly different among fermentations, ranging between 0.37 (MPSC1) and 0.51 g/L (TD). Galacturonic acid is formed during the breakdown of pectin by pectinase enzymes. Galacturonic acid contents were significantly different between the fermentations and ranged from 0.02 g/L (SC1) to 0.05 g/L (MPSC1). This result is consistent with the low pectinase activities of *S. cerevisiae* (Fernández-González et al., 2004) and high pectinase activities of *M. pulcherrima* (Belda et al., 2016).

### 3.3. Volatile compound profile in black currant juice and fermented beverages

A total of 98 volatile compounds were identified from black currant juice (BCJ) and fermented beverages (FBs). Details on the volatile compounds and compound identification are given in Supplementary Table 3. To the best of our knowledge, this is the first report of volatile compounds in black currant beverages that were fermented with the use of different oenological yeasts. Thus, for the interpretation of results we must refer to the results previously reported from grape or fruit wines and juices, respectively. Twenty-six volatile compounds were detected only in the BCJ, whereas 37 were detected only in the FBs, and 34 were detected in both sample types. The identified compounds belonged to acetates (8), fatty acids (8), higher alcohols (20), aldehydes (13), benzenes (2), esters (16), ethers (1), ketones (4), and terpenes (26). Seventy-six volatile compounds with suitable peak shapes were semiquantified in the BCJ and FB samples (Table 1). In addition, the averaged alcoholic beverage (AvB) was averaged from all FB samples. As expected, fermentations changed the volatile profiles significantly compared to the BCJ. Terpenes and esters were the most abundant compound groups in the initial BCJ before fermentation (379 μg/L and 314 μg/L, respectively), whereas higher alcohols and fatty acids were the compounds with the highest concentrations in the AvB (Table 1; 3336 μg/L and 614 μg/L, respectively).

To visualise the correlation between the black currant juice and fermented beverages, a principal component analysis (PCA) was composed of one juice sample, 7 fermented samples, and 76 volatile variables (Fig. 1). The first three PCs explained 84 % of the data variance. BCJ was separated and negatively correlated with the FBs on PC1. On PC2, the *S. bayanus* fermented sample (SB) negatively correlated with the other FBs. The correlation loadings plot shows how certain volatile compounds correlate with the BCJ and others with the FBs. PC3 does not have a major effect on the juice sample, but it separates the *T. delbrueckii* fermented sample (TD) from the other samples and locates the SB with the other FB samples.

Esters are formed by the condensation of the carboxyl group in the acids and the hydroxy group in alcohols. The most important esters for the wine aroma are the ethyl esters of the saturated fatty acids and the acetates of the higher alcohols (Jackson, 2000). The formation of acetate esters is part of the nitrogen metabolism of yeast, where higher alcohols are formed as byproducts from amino acids and esterified with acetyl-CoA by acetyltransferase enzymes (Waterhouse et al., 2016). Ten acetates were identified from the BCJ and FB. Propyl acetate was detected only in the BCJ (Supplementary Table 2). Methyl acetate and ethyl acetate were detected in the juice and fermented beverages. The methyl acetate content was 1.5-fold higher in the averaged fermented beverage (AvB) than in the BCJ. Ethyl acetate was the most abundant acetate in the BCJ (57 μg/L) and AvB (233.1 μg/L) and it formed 88 % of all acetates in the juice sample. The total acetate content increased by as much as 643 % (MPSC1; in average 482 %) during fermentations. A total of 16 esters were detected in the juice and fermented samples. Five of them were only detected in the juice and nine were only detected after fermentations. Generally, the contents of methyl esters decreased during fermentation, and the ethyl esters were formed during fermentation. Only the level of ethyl butanoate did not change during fermentation, whereas methyl propanoate disappeared and the methyl hexanoate level decreased by 86 % (Table 1).

Twenty-one higher alcohols were detected (Supplementary Table 3): (*E*)-2-hexenol was only detected in the juice sample, 15 alcohols only after fermentations, and 5 in both sample types. Higher alcohols in the juice are most likely enzymatically formed degradation products from the corresponding fatty acids (Jung et al., 2017). During alcohol fermentation, higher alcohols are produced from amino acids or sugars by yeast (Ribéreau-Gayon et al., 2006). In addition, aldehydes present in the raw material are reduced to their corresponding higher alcohols during fermentation (Waterhouse et al., 2016). The increase in the total

**Table 1**
Relative concentrations (mean, n = 4)[b] of the volatile compounds identified in the black currant juice, fermented beverages, and averaged fermented beverage.

| No. | Compound[a] | RI | SC1 (μg/L)[b] | SC2 (μg/L)[b] | SB (μg/L)[b] | TD (μg/L)[b] | MPSC1 (μg/L)[b] | MFSC1 (μg/L)[b] | MPSC2 (μg/L)[b] | Juice (μg/L)[b] | Av FB (μg/L)[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Acetates (Ace)* | | | | | | | | | | |
| 1 | Methyl acetate | 499 | 5.8 ± 0.4bc | 6.7 ± 0.6 cd | 3.7 ± 0.2a | 3.9 ± 0.1a | 5.2 ± 1b | 6.4 ± 0.2c | 7.5 ± 0.2d | 3.8 ± 0.3 | 5.6 ± 0.3* |
| 2 | Ethyl acetate | 597 | 212.6 ± 14.1b | 211.4 ± 33.3b | 150.9 ± 24.4a | 236.7 ± 23.8bc | 207.3 ± 35.9ab | 274.8 ± 16c | 335.7 ± 14d | 57.1 ± 3.7 | 233.1 ± 12.2* |
| 3 | Propyl acetate | 709 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 0.9 ± 0.1* | N/D |
| 4 | 2-Methylpropyl acetate | 773 | 16.1 ± 2.2b | 14.2 ± 4.5ab | 6.1 ± 0.3a | 7.7 ± 2.9ab | 5.2 ± 0.7ab | 10 ± 8.5ab | 8.9 ± 4ab | N/D | 9.9 ± 1.1* |
| 5 | Butyl acetate | 813 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 3.5 ± 0.2 | N/D |
| 6 | 3-Methylbutyl acetate | 876 | 78.9 ± 13.8bc | 80.7 ± 7.5c | 132.4 ± 8.6e | 57.9 ± 7.9ab | 48.1 ± 4.5a | 113 ± 5.6de | 96.2 ± 5.8 cd | N/D | 88.8 ± 5.6 |
| 7 | 2-Methylbutyl acetate | 879 | 6.9 ± 1ab | 9.8 ± 1bc | 15.5 ± 0.8de | 5.5 ± 0.4a | 8.8 ± 0.9b | 12.7 ± 0.8 cd | 17 ± 1.3e | N/D | 10.8 ± 1.0* |
| 8 | 2-Phenylethyl acetate | 1268 | 44.4 ± 2.5b | 29.7 ± 1.1ab | 49.6 ± 2.7b | 42.8 ± 2.5ab | 19.6 ± 0.9a | 18.5 ± 1.4a | 21.3 ± 1.7a | N/D | 32.1 ± 2.8* |
| | Tot.Ace | | 364.7 ± 30.0ab | 347.9 ± 45.8a | 358.5 ± 32.1ab | 342.4 ± 19.2a | 294.3 ± 42.5a | 428.8 ± 13.2bc | 482.9 ± 21.6c | 65.0 ± 2.2 | 378.4 ± 62.3* |
| | *Acids (FA)* | | | | | | | | | | |
| 9 | Acetic acid | 604 | 82 ± 56.7 | 93.5 ± 64.7 | 13.1 ± 13.2 | N/D | 100.5 ± 6.3 | N/D | 30.8 ± 35.6 | N/D | 43.1 ± 10.5* |
| 10 | 2-Methyl-propanoic acid | 748 | 18.3 ± 3.7a | 31.0 ± 6.2b | 22.1 ± 4.2ab | 31.3 ± 1.8b | 15.4 ± 1.5a | 13.9 ± 3.9a | 21.4 ± 4.8ab | N/D | 22.8 ± 7.3* |
| 11 | Butanoic acid | 774 | N/D | 17.9 ± 0.2 | 12.1 ± 2.1 | 20 ± 4.1 | 14.9 ± 0 | N/D | N/D | N/D | 7.8 ± 1.7* |
| 12 | 3-Methyl-butanoic acid | 830 | 2.2 ± 0.4 | 6.1 ± 1.4 | 4.8 ± 2.7 | 3.7 ± 1.9 | 3.0 ± 1.7 | 1.8 ± 0.6 | 4.8 ± 2.0 | N/D | 3.9 ± 2.1 |
| 13 | 2-Methyl-butanoic acid | 840 | 5.7 ± 0.8ab | 15.1 ± 4.7bc | 16.2 ± 3.5c | N/D | 4.7 ± 1.4ab | 3.9 ± 0.1a | 8.5 ± 1.7abc | N/D | 8.8 ± 6.6* |
| 14 | Hexanoic acid | 967 | 20.6 ± 2.9abc | 27.1 ± 1.4bcd | 13.8 ± 3.5ab | 13.4 ± 3.8a | 24.6 ± 3.4bc | 46.9 ± 3.6d | 29.5 ± 4.1 cd | N/D | 23.1 ± 2.2* |
| 15 | Octanoic acid | 1162 | 142 ± 15.7bc | 139.4 ± 4.4bc | 99.1 ± 11.2ab | 61 ± 0.6a | 140 ± 17.9bc | 196.2 ± 18.7bc | 155.5 ± 10.7c | N/D | 123.9 ± 10.8* |
| 16 | Decanoic acid | 1357 | 4.1 ± 0.8 | 6.9 ± 2.5 | N/D | 3.7 ± 0.5 | 4.1 ± 0.1 | 7.5 ± 0.3 | 5.6 ± 1.6 | N/D | 5.2 ± 1.9* |
| | Tot.FA | | 533.8 ± 71.4ab | 745.0 ± 54.3c | 606.2 ± 30.6abc | 482.8 ± 74.4a | 570.0 ± 42.3abc | 684.1 ± 107.8bc | 665.0 ± 102.5abc | N/D | 613.7 ± 111.3* |
| | *Higher alcohols (HA)* | | | | | | | | | | |
| 17 | 2-methyl-1-propanol | 615 | 258.9 ± 4.2a | 421.5 ± 17.2c | 467.2 ± 50.3c | 370.4 ± 44.7bc | 264.8 ± 6ab | 433.4 ± 70.2c | 448 ± 52.7c | N/D | 387.8 ± 17.4* |
| 18 | 1-Butanol | 652 | N/D | 3.7 ± 0.3 | N/D | 5.1 ± 1.2 | N/D | 6.2 ± 0.1 | N/D | N/D | 1.7 ± 0.5* |
| 19 | 3-Methyl-1-Butanol | 731 | 1878.1 ± 63.3a | 2397.4 ± 242.5ab | 3140.9 ± 388.2b | 2580 ± 463.8ab | 1847 ± 216.4a | 2443.4 ± 503.7ab | 2622.8 ± 387.8ab | 3.2 ± 0.7 | 2 460.0 ± 103.5* |
| 20 | 2-Methyl-1-Butanol | 735 | 629.2 ± 38.3ab | 750.6 ± 35.7c | 797.7 ± 58.3 cd | 599.6 ± 22.6a | 724.6 ± 20.9bc | 703.2 ± 18.5abc | 887.7 ± 67.1d | N/D | 728.7 ± 21.0* |
| 21 | Pentanol | 765 | 15.4 ± 0.9 | 13.1 ± 4.6 | 14.2 ± 0.8 | 17.5 ± 2.3 | 20.4 ± 0.8 | 18.6 ± 0.8 | 18 ± 3.3 | 17.6 ± 0.9 | 15.1 ± 1.1 |
| 22 | 3-Methyl-2-buten-1-ol | 776 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 50.7 ± 1.1* | N/D |
| 23 | 2,3-Butanediol | 790 | 17.3 ± 1.6ab | 32.9 ± 5.3b | 35.2 ± 9.3b | 10.1 ± 4.3a | 9 ± 4.5a | 8.4 ± 0.2a | 5.2 ± 1.4a | N/D | 17.8 ± 12.3* |
| 24 | Furamethanol | 857 | 2.3 ± 1.1ab | 2.1 ± 0.9a | 2.7 ± 1.2ab | 1.7 ± 0.7a | 3.8 ± 1.8ab | 5.0 ± 0.6b | 3.4 ± 0.9ab | N/D | 1.9 ± 0.5* |
| 25 | (*E*)-2-Hexenol | 867 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 7.1 ± 1.0* | N/D |
| 26 | Hexanol | 868 | 23.8 ± 2.2 | 24.5 ± 3.7 | 23.7 ± 2.1 | 24.4 ± 3 | 21.7 ± 1.7 | 20.4 ± 3.5 | 25.9 ± 3.7 | 6.8 ± 0.6 | 23.7 ± 0.6* |
| 27 | Heptanol | 972 | 12 ± 0.6a | 17.3 ± 1.3a | 28.8 ± 1.4b | 12.6 ± 1.1a | 9.7 ± 0.9a | 9.9 ± 0.3a | 13.2 ± 3.5a | 0.8 ± 0.1 | 14.9 ± 1.5* |
| 28 | 3-(Methylthio)-1-propanol | 981 | 14.6 ± 1.8ab | 14.9 ± 1.4a | 16.4 ± 2.8ab | 23.3 ± 2.7b | 17.8 ± 1.4ab | 10.5 ± 2.8a | 13.5 ± 1.7a | N/D | 15.3 ± 1.1* |
| 29 | 2-Ethyl-1-hexanol | 1030 | 9.3 ± 0.8 | 7.2 ± 4.9 | 8.2 ± 0.6 | 10.4 ± 2.2 | 7.2 ± 0.1 | 6.9 ± 0.3 | 8.1 ± 1.6 | 16.0 ± 4.1 | 8.2 ± 0.4 |
| 30 | Octanol | 1071 | 10.9 ± 1.8ab | 10.8 ± 3ab | 19.6 ± 1.2b | 4.8 ± 0.1a | 13.3 ± 0.5ab | 9.5 ± 0.5ab | 13.2 ± 0.6ab | N/D | 10.3 ± 1.3* |
| 31 | Phenethyl alcohol | 1128 | 544 ± 21.1a | 520.9 ± 10.4a | 733.7 ± 35b | 739.1 ± 36.3b | 510.2 ± 12.5a | 491.7 ± 28.7a | 511.7 ± 28.3a | N/D | 587.7 ± 21.6* |
| 32 | Nonanol | 1172 | 1.6 ± 0.1a | 6.7 ± 1.9bc | 8.2 ± 0c | 7.1 ± 2.4bc | 9.7 ± 0.5c | 7.7 ± 1.5c | 2.7 ± 1.4ab | N/D | 3.2 ± 0.8* |
| 33 | Decanol | 1274 | 4.4 ± 0.1ab | 2.9 ± 1ab | 4.4 ± 0.7bc | 1.3 ± 0.1a | 7.2 ± 1.3c | 3.4 ± 0.2ab | 2.9 ± 0.7ab | N/D | 3.3 ± 1.8* |
| | Tot.HA | | 2621.4 ± 97.3a | 3027.2 ± 158.3ab | 4895.3 ± 378.8c | 3270.4 ± 456.8ab | 2818.1 ± 241.0a | 3431.1 ± 491.4ab | 3614.8 ± 314.7bc | 125.2 ± 23.9 | 3335.9 ± 564.0* |
| | *Aldehydes (Ald)* | | | | | | | | | | |
| 34 | 3-methylbutanal | 642 | 2.3 ± 0.3 | 2 ± 0.4 | 2.9 ± 0.4 | 2.2 ± 0.3 | 2.2 ± 0.3 | 3 ± 0.6 | 2.4 ± 0.7 | N/D | 2.1 ± 0.2* |
| 35 | Pentanal | 689 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 52.6 ± 2.5* | N/D |
| 36 | (*Z*)-2-Pentenal | 753 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | | N/D |

(*continued on next page*)

**Table 1** (*continued*)

| No. | Compound[a] | RI | SC1 (μg/L)[b] | SC2 (μg/L)[b] | SB (μg/L)[b] | TD (μg/L)[b] | MPSC1 (μg/L)[b] | MFSC1 (μg/L)[b] | MPSC2 (μg/L)[b] | Juice (μg/L)[b] | Av FB (μg/L)[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 7.2 ± 1.0* | |
| 37 | Hexanal | 800 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 11.7 ± 0.8 | N/D |
| 38 | Furfural | 836 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 20.4 ± 1.2 | N/D |
| 39 | Heptanal | 902 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 18.1 ± 2.5* | 1.9 ± 0.5 |
| 40 | (*Z*)-2-Heptenal | 960 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 36.5 ± 1.9* | N/D |
| 41 | Benzaldehyde | 971 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 7.7 ± 13 | N/D |
| 42 | Octanal | 1004 | 1.7 ± 0.2a | N/D | N/D | 10.4 ± 2.5b | 7.5 ± 0.2b | 4.9 ± 3.5b | N/D | 16.8 ± 3.8* | 6.6 ± 3.8 |
| 43 | (*Z*)-2-Octenal | 1062 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 16.7 ± 0.5* | N/D |
| 44 | Nonanal | 1107 | 7.5 ± 0.9ab | 6.9 ± 0.2a | 6.9 ± 1ab | 23 ± 7.2c | 10.1 ± 0ab | 10.7 ± 0.9ab | 13.6 ± 2.9bc | 33.4 ± 7.9* | 10.5 ± 1.5 |
| | Tot.Ald | | 11.5 ± 0.8ab | 22.3 ± 6.1abc | 7.3 ± 2.6a | 37.7 ± 10.6c | 26.5 ± 0.1bc | 24.3 ± 4.7abc | 25.0 ± 10.0bc | 205.9 ± 31.3* | 21.2 ± 11.2 |
| | *Benzenes* | | | | | | | | | | |
| 45 | Vinyl benzene (Styrene) | 898 | 2.5 ± 0.5a | 3.9 ± 0.8abc | 4.2 ± 0.5ab | 4 ± 0.9abc | 6.1 ± 0.9c | 5.2 ± 0.3bc | 3.5 ± 0.3abc | N/D | 3.9 ± 0.3* |
| | *Esters (Es)* | | | | | | | | | | |
| 46 | Methyl propanoate | 616 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 5.6 ± 1.3* | N/D |
| 47 | Ethyl propanoate | 706 | 1.7 ± 0.1 | 1.2 ± 0.4 | 2.5 ± 0.3 | 3.1 ± 0. | 1 ± 0.1 | 2.5 ± 0.3 | 2.5 ± 0.2 | N/D | 1.6 ± 0.3* |
| 48 | Methyl butanoate | 717 | 31.2 ± 4.5a | 32.1 ± 3.2a | 30.7 ± 2.9a | 34.9 ± 3.7a | 54.9 ± 6.1b | 54.1 ± 2.4b | 52.7 ± 4b | 283.1 ± 23.5* | 40.0 ± 2.2 |
| 49 | Ethyl butanoate | 800 | 16.1 ± 2.7a | 15.9 ± 1.8a | 16.4 ± 1.1a | 16.4 ± 2.3a | 24.3 ± 1.5b | 31.1 ± 1.3b | 21.9 ± 1.7c | 19.2 ± 1.9 | 19.5 ± 1.1 |
| 50 | Methyl hexanoate | 924 | 1.5 ± 0.3ab | 1.2 ± 0.2a | 1.3 ± 0.1ab | 1.6 ± 0.3ab | 1.9 ± 0.5ab | 2.5 ± 0.5bc | 3.2 ± 0.9b | 11.5 ± 1.6* | 1.9 ± 0.8 |
| 51 | Ethyl hexanoate | 999 | 11.3 ± 2.3bc | 9.1 ± 1.1ab | 13.1 ± 0.5 cd | 7.8 ± 1a | 21.1 ± 2.5e | 19.8 ± 0.4e | 15.4 ± 0.7d | N/D | 13.1 ± 0.9* |
| 52 | Ethyl octanoate | 1195 | 70.3 ± 13.5b | 69.4 ± 7.2b | 122.9 ± 5.7e | 33.2 ± 5.9a | 103 ± 12.4de | 92.6 ± 5.2 cd | 80.3 ± 7.2bc | N/D | 79.5 ± 5.7* |
| 53 | Ethyl 9-deconoate | 1388 | 6.6 ± 1.8b | 5.7 ± 0.7b | 16.2 ± 1.1c | N/D | N/D | N/D | 1.6 ± 0.1a | N/D | 7.6 ± 6.0* |
| 54 | Ethyl decanoate | 1595 | 64.2 ± 5.4a | 41 ± 5.4a | 174 ± 13.7c | 38.9 ± 8.9a | 108.1 ± 13.7b | 66.2 ± 6.6a | 56.4 ± 3.1a | N/D | 73.9 ± 10.0* |
| 55 | 3-Methylbutyl octanoate | 1449 | 3.6 ± 0.4a | 2.5 ± 0.5a | 8.7 ± 3.4b | 3.2 ± 0.3a | 4 ± 0.4a | 4 ± 0.3a | 3.8 ± 0.3a | N/D | 4.1 ± 0.5* |
| 56 | Ethyl dodecanoate | 1595 | 45.4 ± 3.b | 25.4 ± 1.6a | 68.1 ± 5.5c | 17.6 ± 3.3a | 91.3 ± 8.6d | 65.3 ± 4.9c | 49.3 ± 1.7a | N/D | 37.3 ± 5.8* |
| 57 | 3-Methylbutyl pentadecanoate | 1647 | 11.4 ± 1c | 5.8 ± 0.6ab | 14.1 ± 0.7c | 3.2 ± 2.3a | 5.1 ± 1ab | 6.4 ± 0.4ab | 4.8 ± 0.3ab | N/D | 7.5 ± 0.8* |
| | Tot.Es | | 243.2 ± 58.7ab | 200.0 ± 35.4a | 465.6 ± 26.3d | 147.9 ± 47.1a | 412.2 ± 51.0 cd | 322.2 ± 34.7bc | 238.0 ± 13.8ab | 314.3 ± 30.7 | 278.8 ± 114.4 |
| | *Ethers* | | | | | | | | | | |
| 58 | 3-Ethoxy-1-propanol | 842 | N/D | N/D | N/D | 31.6 ± 6b | 5.7 ± 0.5a | 3.8 ± 0a | N/D | N/D | 8.8 ± 15.1* |
| | *Ketones (Ke)* | | | | | | | | | | |
| 59 | 2-Pentanone | 677 | 2 ± 0.1 | 1.4 ± 0.3 | N/D | 1 ± 0.1 | N/D | N/D | N/D | 3.3 ± 0.2* | 0.4 ± 0.1 |
| 60 | 1-Octen-3-one | 979 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 17.1 ± 1.7* | N/D |
| 61 | 6-Methyl-5-hepten-2-one | 988 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 35.6 ± 1.4* | N/D |
| | Tot.Ke | | 2.0 ± 0.1 | 1.4 ± 0.3 | N/D | 1.0 ± 0.1 | N/D | N/D | N/D | 56 ± 3.1* | 0.4 ± 0.7 |
| | *Terpenes (Te)* | | | | | | | | | | |
| 62 | *α*-pinene | 946 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 22.1 ± 2.3* | N/D |
| 63 | Camphene | 964 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 11.7 ± 1.1* | N/D |
| 64 | *β*-Myrcene | 993 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 10.8 ± 2.7 | N/D |
| 65 | *δ*-3-Carene | 1024 | N/D | N/D | N/D | N/D | N/D | N/D | N/D | 58.6 ± 4.8* | N/D |
| 66 | *o*-Cymene | 1036 | 5.2 ± 0.7 | 5.3 ± 0.3 | 8.4 ± 1.2 | 7 ± 0.9 | 7 ± 1.9 | 6.1 ± 1.2 | 6.6 ± 0.4 | 24.1 ± 22.7* | 5.2 ± 0.6 |
| 67 | D-Limonene | 1040 | 7.3 ± 1.3 | 8.4 ± 0.4 | 8.4 ± 1.9 | 9.3 ± 0.2 | 5.7 ± 2 | 3 ± 0.4 | 7.1 ± 0.5 | 22.2 ± 2.4* | 6.0 ± 0.7 |
| 68 | 1,8-Cineol | 1045 | 11.8 ± 0.2b | 5.0 ± 1.0a | 16.7 ± 3.2c | 3.5 ± 1.0a | 5.6 ± 1.2a | 4.9 ± 0.3a | 5.6 ± 0.3a | 11.8 ± 0.0* | 7.0 ± 0.3 |
| 69 | (*Z*)-Linalooloxide | 1085 | 6.8 ± 0.3 | 7.3 ± 1.4 | 7.9 ± 0.9 | 8.0 ± 1.0 | 8.7 ± 0.6 | 6.7 ± 1.3 | 7.0 ± 0.3 | 9.2 ± 1.0 | 7.5 ± 1.0 |
| 70 | Linalool | 1104 | | | | 10.4 ± 0.3a | | 19.1 ± 6.7b | | | 13.9 ± 1.0 |

(*continued on next page*)

**Table 1** (*continued*)

| No. | Compound[a] | RI | SC1 (μg/L)[b] | SC2 (μg/L)[b] | SB (μg/L)[b] | TD (μg/L)[b] | MPSC1 (μg/L)[b] | MFSC1 (μg/L)[b] | MPSC2 (μg/L)[b] | Juice (μg/L)[b] | Av FB (μg/L)[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 17.2 ± 1.5ab | 12.8 ± 2.8ab | 12.6 ± 4.1ab | | 15.4 ± 1.1ab | | 14.8 ± 1.4ab | 16.3 ± 2.5 | |
| 71 | (*E*)-Linalool oxide acetate (pyranoid) | 1296 | 5.1 ± 0.2a | 6.0 ± 0.6ab | 5.3 ± 0.9ab | 6.6 ± 0.3b | 4.7 ± 0.5a | 5.8 ± 0.5ab | 5.2 ± 0.5a | 9.6 ± 0.7* | 5.6 ± 0.7 |
| 72 | Bornyl acetate | 1307 | 5.7 ± 1.1a | 5.9 ± 0.6a | 11.7 ± 0.4b | 7.3 ± 5ab | 8.3 ± 0.4ab | 12.6 ± 0.5b | 9 ± 0.9ab | 43.5 ± 4.1* | 8.5 ± 0.6 |
| 73 | *β*-Damascenone | 1410 | 5.5 ± 0.5ab | 5.3 ± 0.1ab | 5.6 ± 0.3ab | 6 ± 0.5b | 5.3 ± 0.7ab | 4.8 ± 0.4a | 5 ± 0.7ab | 21.3 ± 2.2* | 5.4 ± 0.1 |
| 74 | Caryophyllene | 1464 | 14.7 ± 1.9ab | 11.6 ± 0.5a | 38.7 ± 1.8e | 22.8 ± 1.8d | 17.3 ± 0.1bc | 14.9 ± 0.6abc | 18.3 ± 1.1c | 117.0 ± 1.8* | 20.1 ± 1.8 |
| 75 | *α*-Humulene | 1498 | 4.6 ± 1.2ab | 3.4 ± 0.6a | 10.1 ± 0.5c | 5.4 ± 0.5b | 4.5 ± 0.1ab | 3.6 ± 0.1a | 4.6 ± 0.3ab | 31.0 ± 2.0* | 5.3 ± 0.5 |
| | Tot.Te | | 62.0 ± 3.8a | 55.5 ± 7.1a | 104.1 ± 3.4c | 72.3 ± 7.5ab | 86.0 ± 3.6bc | 83.6 ± 16.0b | 67.1 ± 7.6ab | 378.6 ± 32.2* | 74.7 ± 17.5 |

[a] Compounds identified in the Supplementary Table 3. Different letters at the same row indicates statistical difference ($p < 0.05$) with one-way ANOVA using Tukey's test. [b] Relative concentration (μg/L, mean, n = 4) collected from the headspace using HS-SPME calculated by comparison of the peak areas with that of the internal standard 2-octanol (50 ng/100 μL) with a response factor of 1. * Statistical difference between the black currant juice and averaged fermented beverage. SC *S. cerevisiae*, SB *S. bayanus*, TD *T. delbrueckii*, MPSC sequential fermentation with *M. pulcherrima* and *S. cerevisiae*, MFSC sequential fermentation with *M. fructicola* and *S. cerevisiae*.

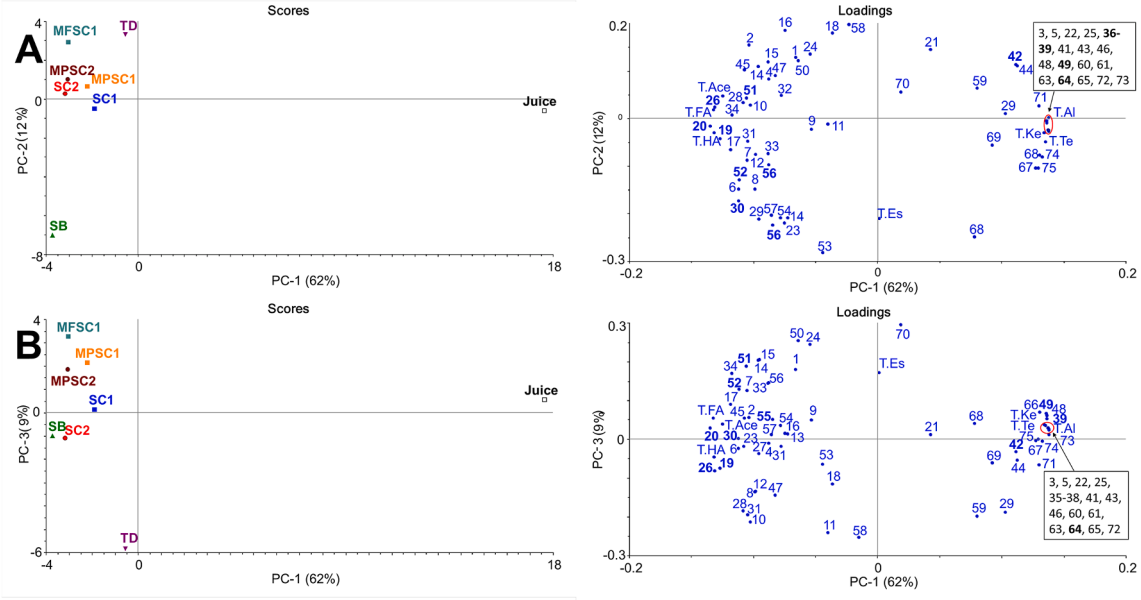


**Fig. 1.** Principal component analysis of average values of biological replicates of the black currant juice and fermented beverages (n = 8) and chemical variables (n = 75) with three components, 1) PC1 vs. PC2, 2) PC1 vs. PC3. □ black currant juice, ■ (blue) SC1 *S. cerevisiae* 1, ● SC2 (red) *S. cerevisiae* 2, ▲(green) SB *S. bayanus*, ▼ (purple) TD *T. delbrueckii*, ■ (orange) MPSC1 *M. pulcherrima* + *S. cerevisiae* 1, ■ (teal) MFSC1 *M. fructicola* + *S. cerevisiae* 1, ● (brown) MPSC2 *M. pulcherrima* + *S. cerevisiae* 2. Numbers and abbreviations of the variables refer to Table 1, and bolded numbers were the most contributing compounds observed in the GC-O analysis.

higher alcohols in the AvB (3336 μg/L) was 256 % compared to that in the BCJ (125 μg/L). Pentanol and 2-ethyl-1-hexanol were the only higher alcohols whose contents did not significantly change during fermentation (Table 1).

Thirteen aldehydes were detected, of which eight were only in the juice and six were in both the juice and fermented beverages (Table 1). Pentanal, heptanal, and nonanal were the major aldehydes in the BCJ. Jung et al. (2017) reported contrary results in three fresh black currant cultivars. They observed that aldehydes composed of six carbons were the major aldehydes in the studied black currant cultivars. In addition, they compared the volatile composition of fresh and freeze-stored black currants. They observed that almost all six-carbon higher alcohols and aldehydes disappeared after nine months of freeze storage, whereas other aldehydes, such as nonanal and decanal, were not affected. The low concentrations of aldehydes composed of six carbons are potentially the result of freeze storage of the black currants. In addition, changes in the volatile composition during freeze storage may indicate different sensory properties for the fermented black currant beverages those prepared from fresh black currants compared to ones prepared from frozen black currants. After fermentations, the total aldehyde content was only 3.5–12.9 % of the initial aldehyde content in the juice sample. Furfural was not found in the non-pasteurized black currant juice (data not shown), but it appeared after pasteurisation. It was also detected after fermentations in trace amounts. Mikkelsen and Poll (2002) and Varming et al. (2004) reported furfural appearing after the pasteurisation of the black currant juice. Formation of furfural may occur via three pathways: the Maillard reaction, degradation of ascorbic acid, and decomposition of pentoses in acidic media after water elimination. Furfural can add baked fragrance to wine (Jackson, 2000).

Fatty acids are produced as byproducts in the fatty acid metabolism of yeast during alcoholic fermentation (Waterhouse et al., 2016). In our study, eight fatty acids were identified after fermentations, but not from the juice. Three of them were branched-chain fatty acids, and five were straight-chain fatty acids (Supplementary Table 3).

A total of 23 terpenes were detected in the samples (Supplementary Table 3). All terpenes were detected in the juice and 14 in the fermented beverages. The total terpene content decreased by approximately 80 % during fermentation (Table 1). The highest decrease was observed for $\beta$-caryophyllene and $\alpha$-humulene, both by 83 %, and the lowest decrease was observed with linalool (15 %). The disappearance of the terpenes may have resulted from acid-catalysed hydrolysis or the rearrangement of the precursors. For example, linalool and $\alpha$-terpineol may be rearranged to 1,8-cineol *via* hydrolysis and intramolecular cyclisation (Waterhouse et al., 2016). 1,8-Cineol has been reported to highly contribute to the black currant aroma (Jung et al., 2017).

Black currant juice (BCJ), *S. bayanus* (SB) and *T. delbrueckii* (TD) fermented beverages (FBs) were selected for GC-O analysis based on the differences observed during the analysis of volatile compounds. Both the nasal impact factor (NIF) and square of nasal impact factor (SNIF) were calculated from the evaluations (n = 10; Table 2). A total of 51 compounds contributing to the aroma were detected and described by GC-O by two or more assessors (NIF $\geq$ 40 %; Table 2). Thirty-three detected scents were described for the compounds detected by GC-FID and identified with GC–MS. The odour descriptions provided by assessors and external standard compounds were used to validate the GC-O identifications.

BCJ had the highest count of the detected compounds (39) in the GC-O analysis. Twenty-eight compounds were detected in the FBs, of which 22 were detected in both FBs. Only 11 compounds were detected in all samples (Table 2). The SNIF values had great differences between the samples. Almost all SNIF values were notably lower in the FBs than in the BCJ, but in a few cases, the SNIF value was higher in at least one FB.

**Table 2**
Volatile compounds identified with GC-O in black currant juice and fermented beverages.

| Compound | RI[a] | RI lit. | Identification methods | BCJ[c] NIF % | BCJ[c] SNIF | SB[c] NIF % | SB[c] SNIF | TD[c] NIF % | TD[c] SNIF | Description[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| n.i | 542 | | | 50 | 106 | | | | | Sweat |
| n.i | 556 | | | | | | | 50 | 122 | Alcohol, fresh, sweet |
| 2,3-Butanedione | 597 | 597[3] | STD, RI | | | 40 | 16 | | | Butter, sweet, soft toffee |
| 2-Methyl butanal | 661 | 660[3] | STD, RI | 40 | 8 | 40 | 16 | | | Musty |
| n.i | 669 | | | 60 | 170 | | | | | Roasted, stuffy, urine |
| Propyl acetate | 704 | 708* | STD, RI | 40 | 32 | | | | | Green, grass, fresh |
| n.i | 729 | | | 50 | 114 | | | | | Sweat, stinky, musty |
| 3-Methyl/2-methyl butanol | 745 | 736[3] | RI, MS | | | 60 | **354** | 70 | **332** | Musty, pungent, rancid |
| Pentanol | 766 | 771[2] | STD, RI | | | 40 | 64 | 60 | 220 | Sweet, fruity, candy |
| n.i | 786 | | LIT | 40 | 96 | | | | | Plastic, pungent, sweet |
| Hexanal | 805 | 809[1] | RI | 50 | 112 | | | | | Grass, green, leaf |
| Ethyl butanoate | 808 | 808[1] | LIT | 50 | 116 | 70 | 200 | 50 | **312** | Sweet, candy, fruit |
| n.i | 824 | | | 70 | 194 | | | | | Stuffy, roasted, mushroom |
| 3-Methylbutanoic acid | 831 | 837* | STD, RI | | | 60 | 192 | 40 | 80 | Solvent, pungent, chemical |
| Methyl 3-hydroxy-butanoate | 857 | 858* | MS, RI | 50 | 178 | 60 | 124 | 50 | 188 | Sweet, fruity, floral |
| 3-Hexe-1-ol | 867 | 856[2] | STD, RI | 40 | 64 | | | | | Sweet, fruity, rhubarb |
| n.i | 869 | | | | | | | 50 | 280 | Popcorn, roasted, baked |
| 1-Hexanol | 872 | 870[3] | STD, RI | 60 | 164 | 80 | **262** | 70 | **364** | Urine, musty, baking, cheese |
| 3-Methylbutyl acetate | 882 | 875[2] | STD, RI | | | 50 | 114 | 50 | 58 | Fruit, pear, sweet |
| Heptanal | 906 | 902[3] | STD, RI | 60 | **382** | | | | | Leaf, green, flower |
| n.i | 908 | | | 50 | 204 | | | | | Mushroom, mould, earth |
| Ethyl 2-hydroxybutanoate | 909 | 910* | MS, RI | | | 50 | 68 | | | Sweet, floral, green |
| Methional | 917 | 907[3] | STD, RI | 50 | 264 | 40 | 16 | 40 | 16 | Potato, cheese, musty |
| n.i | 925 | | | 60 | **414** | 60 | 238 | 50 | **286** | Sweat, pungent, musty |
| Heptanol | 970 | 970[3] | STD, RI | 50 | 130 | 50 | 26 | 60 | 188 | Musty, pungent, spoiled |
| 1-Octen-3-one/1-octen-3-ol | 985 | 979/980 | MS, RI | 70 | **400** | 60 | 202 | 50 | **294** | Mushroom, earthy, pungent |
| $\beta$-Myrcene | 990 | 993[1] | STD, RI | 70 | **518** | 40 | 80 | 60 | 246 | Raw carrot, metallic, chemical |
| Ethyl hexanoate | 1005 | 1000[3] | LIT | | | 60 | **304** | 50 | 182 | Sweet, fruit, pineapple |
| Octanal | 1006 | 1003[3] | STD, RI | 70 | **362** | | | | | Citrus fruit, green, lemongrass |
| 1,8-Cineol | 1034 | 1034[1] | LIT | 40 | 16 | 40 | 8 | | | Eucalyptus, mint, pastille |
| (*E*)-Ocimene | 1051 | 1050[1] | LIT | 40 | 64 | | | | | Chemical, pungent, sweet |
| (*E*)-2-Octenal | 1063 | 1060[3] | STD, RI | 50 | 162 | | | | | Lemongrass, green, grass |
| n.i | 1102 | | MS, RI | 60 | 162 | 50 | 82 | 40 | 72 | Pungent, solvent, green |
| Linalool | 1106 | 1103[1] | LIT | 50 | 220 | 60 | 178 | 50 | 222 | Fresh, leaf, green |
| Rose oxide | 1108 | 1109[1] | STD, RI | 60 | 120 | 40 | 8 | 50 | 92 | Fresh, rose, floral |
| Phenylethyl alcohol | 1126 | 1124[3] | MS, RI | | | 50 | 42 | 40 | 8 | Rose, floral, perfume |
| (*E*)-2-Nonenal | 1161 | 1161[3] | LIT | 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 | 1189 | 1188[1] | LIT | 40 | 48 | | | 50 | 76 | Herbal, grass, earth |
| Terpinen-4-ol | 1194 | 1183[1] | MS, RI | 60 | 252 | 50 | 102 | 50 | 156 | Herbal, pungent, bell pepper |
| Terpineol | 1201 | 1198[1] | MS, RI | 50 | 130 | | | | | Liquorice, anise |
| Ethyl octanoate | 1213 | 1198[2] | STD, RI | | | 40 | **344** | 40 | 160 | Sweet, passion fruit, candy |
| n.i | 1265 | | MS, RI | 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 |
| $\beta$-damascenone | 1398 | 1386[3] | MS, RI | 40 | 8 | | | | | Berry-like, rowanberry, honey |
| 3-methylbutyl octanoate | 1427 | 1433* | MS, RI | | | 40 | **322** | 50 | 186 | Fruit, berry-like, honey |
| n.i | 1428 | | | 60 | 346 | | | | | Sweet, berry-like, honey |
| n.i | 1455 | | | 50 | 164 | | | | | Vanilla, sweet |

[a] Linear retention indices; [b] Three most frequently given description by panellists (n = 2 × 5); [1] (Marsol-Vall et al., 2019); [2] (Liu et al., 2019); [3] (Babushok et al., 2011); * NIST14 database; LIT identification by RI and descriptors in Marsol-Vall et al. (2019); MS identification based only on comparison of identification from MS data, aroma descriptors, and RI to literature; bold numbers are the five highest SNIF values in the sample. [c] BCJ black currant juice, SB *S. bayanus* fermented, TD *T. delbrueckii* fermented.

For example, the SNIF value of ethyl butanoate was 1.7 times higher in the SB and 2.7 times higher in the TD than in the BCJ.

Fourteen compounds were only detected in the FBs, two of which were only found in the TD beverage, two unidentified compounds with RIs of 556 (described as alcohol, fresh, sweet) and 869 (popcorn, roasted, baked), and two only in the SB beverage, 2,3-butanedione and 2-hydroxy ethyl butanoate.

According to the SNIF values (Table 2), the five most potent aroma compounds contributing to the studied black currant juice were $\beta$-myrcene, an unidentified compound with RI 925 (sweat, pungent, musty), 1-octen-3-one/1-octen-3-ol, 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 those in the TD fermented beverage were hexanol, 3-methyl/2-methyl butanol, ethyl butanoate, 1-octen-3-one/1-octen-3-ol, and one unidentified compound with RI 925.

### 3.4. Impacts of the yeast strains on volatile compounds

Sixty volatile compounds were quantified from the FBs, of which 47 were significantly different between the yeast strains (Table 1). Higher alcohols were the most abundant volatile group, contributing 66.1–74.6 % of the total volatile compounds. The second most abundant group was fatty acids (10.6–16.0 %) and the third was acetate esters (6.3–9.4 %).

To visualise the correlations between the fermented black currant beverages, another PCA was composed of 7 fermented beverage samples and 58 volatile variables (Fig. 2). The first three PCs explain 76 % of the data variance. PC1 clearly separates the sequential fermentations from the beverages fermented with the *Saccharomyces* yeasts but not from the TD sample. In addition, SB was separated from the other *Saccharomyces* fermented samples (SC1 and SC2) on PC1. It also shows a negative correlation with the TD on PC1 and PC2. The TD negatively correlates with sequential fermentations on the PC2. SC1 and SC2 are located near each other, indicating their similarity in fermentation behaviour. Few volatile variables, such as acetic acid (variable 9), nonanol (32), and *Z*-linaloloxide (69), exhibit positive correlations with SC beverages on PC1 and 2. On PC3, only acetic acid and 2-methylpropyl acetate (4) positively correlated with SC1 and SC2. Fifteen volatile variables, such as 2,3-butanediol (23), 1,8-cineol (68), heptanol (27), and 3-methylbutyl acetate (6), are clustered near SB. PC3 better separates single yeasts from each other and clear species differences can be seen. The PCA clearly shows the importance of *M. pulcherrima* (MP) on sequential fermentations: the MPSC1 and MPSC2 are located closer to one another than to sequential fermentation with *M. fructicola* and *S. cerevisiae* 1 (MFSC1). Surprisingly, PCA3 separates MPSC1 and MPSC2 from each other, locating MPSC2 close to SCs and MFSC1 and MPSC1 close to the TD. On PC3, straight-chain fatty acids, such as hexanoic, octanoic, and decanoic acids, as well as ethyl acetate were positively correlated with MPSC2. All of these compounds may have a negative impact on the sensory properties at high concentrations. Methyl butanoate (48), ethyl butanoate (49), ethyl hexanoate (51), pentanol (21), nonanal (44), octanal (42), and *Z*-linalooloxide (69) relocate to PC3 to correlate with MPSC1 and MPSC2. These compounds may have a positive effect on the sensory properties of the fermented beverages.

A wide range of the total higher alcohols was determined after fermentations: SB fermentation resulted in the highest (4154 μg/L) and SC1 fermentation resulted in the lowest total higher alcohol content (2667 μg/L; Table 1). Interestingly, sequential fermentation with 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. 3-Methyl-1-butanol and 2-methyl-1-butanol were the most abundant higher alcohols after every fermentation. Prior et al. (2019) reported sequential fermentation of *M. pulcherrima* and *S. cerevisiae* to produce more 3-methyl-1-butanol, 2-methyl-1-propanol, and phenethyl alcohol than single-yeast fermentation with *S. cerevisiae*. In our study, similar results were observed: the sequential fermentation with the MPSC1 produced slightly more 2-methyl-1-propanol than the SC1, and the MPSC2 produced more 2-methyl-1-propanol and 3-methyl-1-butanol compared to the SC2. In both cases, the single yeast fermentation with *S. cerevisiae* produced more phenethyl alcohol. The amino acid profile has an effect on the contents of higher alcohols. For example, 2-methyl-1-butanol, 3-methyl-1-butanol, and 2-phenylethanol are degradation products of isoleucine, leucine, and phenylalanine, respectively (Waterhouse et al., 2016). In addition, oenological yeast may have a significant effect on the contents of higher alcohols (Prior et al., 2019).

The total acetate contents varied between 294 μg/L (MPSC1) and 483 μg/L (MPSC2; Table 1). SC1 had a slightly higher total acetate

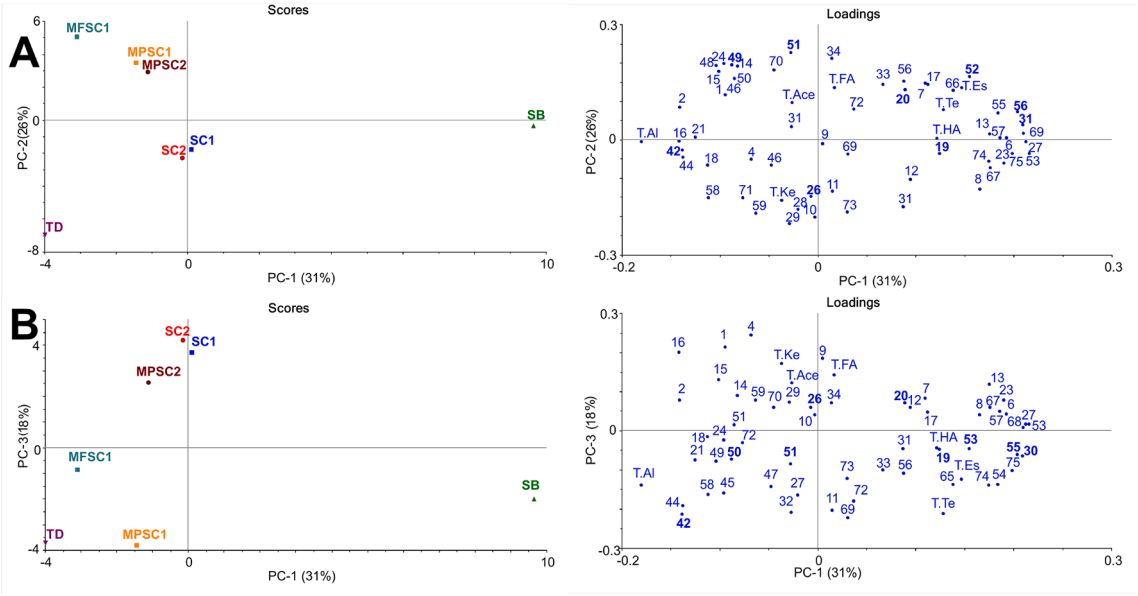


**Fig. 2.** Principal component analysis of average values of biological replicates of fermented black currant beverages (n = 7) and chemical variables (n = 58) with three components, A) PC1 vs. PC2, B) PC1 vs. PC3. ■ (blue) SC1 *S. cerevisiae* 1, ● (red) SC2 *S. cerevisiae* 2, ▲ (green) SB *S. bayanus*, ▼ (purple) TD *T. delbrueckii*, ■ (orange) MPSC1 *M. pulcherrima* + *S. cerevisiae* 1, ■ (teal) MFSC1 *M. fructicola* + *S. cerevisiae* 1, ● (brown) MPSC2 *M. pulcherrima* + *S. cerevisiae* 2. Numbers and abbreviations of the variables refer to Table 1, and bolded numbers were the most contributing compounds observed in the GC-O analysis.

content (365 μg/L) than SC2 (348 μg/L). Ethyl acetate was the most abundant acetate in the FBs. The highest concentration of ethyl acetate was observed in MPSC2 (336 μg/L) and the lowest was observed in SB (151 μg/L). Duarte et al. (2010) studied the effects of *S. cerevisiae* and *S. bayanus* on raspberry wine. They reported that *S. cerevisiae* yeasts produced at least 1.4-fold higher amounts of ethyl acetate than *S. bayanus*. This result is consistent with our results, where SC fermentations resulted in a content of ethyl acetate 0.7-fold higher than that from SB fermentation. The excess concentration of ethyl acetate may have a negative impact on wine sensory properties (Lambrechts & Pretorius, 2000). Interestingly, the sequential fermentations produced 2.3–2.7-fold lower levels (18.5–21.3 μg/L) of phenethyl acetate than the highest level (49.6 μg/L) produced by SB.

Ethyl esters were the main esters in the FBs, contributing 79–96 % of all esters (Table 1). The total ester contents varied between 148 μg/L (TD) and 466 μg/L (SB). Interestingly, SB produced all esters at significantly higher levels than TD. Fermentation with TD had the lowest production of esters among all fermentations, although some studies have reported a high capacity of TD for producing esters. Prior et al. (2019) and Liu et al. (2019) reported similar results to ours. Liu et al. used two *T. delbrueckii* strains, of which one exhibited ester production performance similar to that shown in our study. Ethyl octanoate and ethyl decanoate were the most abundant esters in all FBs. The highest concentrations were observed in the SB beverage (123 μg/L and 174 μg/L, respectively), and the lowest were observed in the TD beverage (33 μg/L and 39 μg/L, respectively). The sequential fermentations with MP resulted in significantly higher ester contents than the corresponding single-yeast fermentations with *S. cerevisiae* yeasts, whereas sequential fermentation with MF resulted in slightly lower levels.

Fatty acids were observed in a wide concentration range in the fermented beverages: the lowest level was in the beverage fermented with TD (446.3 μg/L) and the highest was in those fermented with SC2 (721.9 μg/L; Table 1)). Both straight-chain and branched-chain fatty acids were detected after all fermentations (Supplementary Table 3). The highest concentration of branched-chain fatty acids was observed in SC2 (30.6 μg/L) and the lowest was observed in SC1 (2.2 μg/L). The MPSC2 beverage had the highest amount of straight-chain fatty acids (250.4 μg/L) and the TD beverage had the lowest concentration (94.8 μg/L). Comitini et al. (2011) reported significantly lower contents of fatty acids when *M. pulcherrima* was used in mixed fermentation with *S. cerevisiae*, which is not consistent with our results. In all FBs, the fatty acid contents were so low that they may not have any effect on the flavour properties.

3-Ethoxy-1-propanol was the only identified ether compound in the FBs. It was detected only in the TD, MPSC1, and MFSC1. The 3-ethoxy-1-propanol concentration in the TD was 5.5–8.3 fold higher than that in the other FBs (Table 1). This is consistent with results reported by Velazquez et al. (2015), who compared *T. delbrueckii* yeast-fermented wines with *S. cerevisiae*-fermented wines. They observed a significantly higher amount of 3-ethoxy-1-propanol in the *T. delbrueckii*-dominant wines than in the *S. cerevisiae*-dominant wines. This result indicates that the *Saccharomyces*-yeast strains did not produce 3-ethoxy-1-propanol but the non-*Saccharomyces* yeasts did.

### 3.5. Sensory quality of fermented black currant beverages

*S. cerevisiae* 1 (SC1), *S. bayanus* (SB), *T. delbrueckii* (TD), and the beverages produced by sequential fermentation with *M. pulcherrima* or *M. fructicola* and *S. cerevisiae* 1 (MPSC1 and MFSC1, respectively) were subjected to descriptive analysis. All FB samples were described as intensely sour. Three-way ANOVA showed statistically significant sample effects only for the black currant odour ($p < 0.05$) and musty odour ($p < 0.01$). Further statistical analysis with one-way ANOVA with a subsequent LSD test showed statistically significant differences ($p < 0.05$) in the black currant odour between SB and TD beverages (Table 3), with TD reaching an intensity of 5.6 and SB reaching 4.6. Musty odour was significantly different between SB beverages (intensity of 4.3) and all of the other beverages (intensities of 2.1–2.6). In addition to these odour attributes, the SB and MFSC1 beverages had the highest viscosities (3.8 and 3.7, respectively), which were significantly higher than those of the TD beverage (3.1).

The partial least square regression (PLS; Fig. 3) was constructed with the fermented beverage samples (n = 5) used in the sensory evaluations, all chemical variables, their total amounts and sugar to acid ratio (X, n = 78), and the sensory attributes (Y, n = 12) for detected compounds contributing to different sensory characteristics (Fig. 3). The first three PLS factors explained 85 % (X) and 91 % (Y) of the variance in the data. Factor-1 divides *Saccharomyces*-fermented beverages (SC1 and SB) from non-*Saccharomyces* (TD) and sequentially fermented beverages (MPSC1 and MFSC1). All samples were described as intensively sour, and the PLS regression model exhibited a clear correlation between the sour taste and the total intensity of flavour. In addition, hexanoic acid (variable 14), octanoic acid (15), and linalool (70) clearly correlate with the total flavour intensity. Citric acid and the total organic acid content are located close to the sour taste, indicating their contribution to the sour taste. Sweet taste is negatively correlated with sugars on factor-2, indicating a strong contribution to this attribute by some other compounds. However, sweet taste, glucose, fructose, and the total sugar content were positively correlated with factor-3 (data not shown).

SB fermented beverage positively correlates with the musty odour. The SB sample formed a cluster with the musty odour and certain volatile compounds. Potential compounds contributing to the musty odour were heptanol (variable 27), octanol (30), 2-methyl butanoic acid (13), and 2,3-butanediol (23). However, the source of mustiness may also be compounds whose contents were not determined in this study, because the detection threshold of many musty compounds is only a few nano- or micrograms (Callejón et al., 2016). Callejón et al. (2016) reported that 1-octen-3-ol was one of the most important compounds contributing to

**Table 3**
Results of sensory evaluations of the fermented black currant beverages.

| | Odour properties | | | | | Mouthfeel | | Taste properties | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | O_Total intensity | O_Blackcurrant | O_Sweet | O_Sour | O_Musty | M_Viscosity | M_Astringency | F_Total intensity | F_Blackcurrant | T_Sweet | T_Bitter | T_Sour |
| SC1 | 6.0 ± 1.5 | 5.0 ± 1.8 | 3.5 ± 1.6 | 4.4 ± 2.0 | 2.5 ± 1.8a | 3.6 ± 1.3 | 4.7 ± 2.0 | 7.5 ± 1.4 | 5.8 ± 1.9 | 2.2 ± 1.0 | 4.5 ± 2.1 | 7.0 ± 1.6 |
| SB | 6.2 ± 1.3 | 4.5 ± 1.7a | 3.5 ± 1.5 | 4.6 ± 1.8 | 4.3 ± 2.2b | 3.8 ± 1.3b | 5.0 ± 2.0 | 7.1 ± 1.6 | 5.6 ± 1.5 | 2.5 ± 1.1 | 5.0 ± 2.0 | 6.6 ± 1.7 |
| TD | 6.2 ± 1.1 | 5.6 ± 1.8b | 4.1 ± 1.5 | 3.8 ± 1.3 | 2.1 ± 1.5a | 3.1 ± 1.3a | 5.0 ± 2.0 | 7.2 ± 1.4 | 6.1 ± 1.5 | 2.5 ± 1.1 | 5.0 ± 1.7 | 6.5 ± 1.6 |
| MPSC1 | 5.9 ± 1.0 | 5.2 ± 1.7 | 3.9 ± 1.6 | 4.4 ± 1.5 | 2.6 ± 1.8a | 3.3 ± 1.2a | 4.7 ± 2.1 | 7.3 ± 1.3 | 6.0 ± 1.8 | 2.4 ± 1.1 | 4.6 ± 2.0 | 6.6 ± 1.7 |
| MFSC1 | 6.1 ± 1.6 | 5.3 ± 1.4 | 3.7 ± 1.6 | 4.0 ± 1.4 | 2.3 ± 1.6a | 3.7 ± 1.2 | 4.7 ± 2.0 | 7.4 ± 1.4 | 6.2 ± 1.6 | 2.6 ± 1.2 | 4.5 ± 1.5 | 7.1 ± 1.7 |

Different letters at same column indicates statistical difference ($p < 0.05$) with one-way ANOVA using LSD test. SC1 *S. cerevisiae* 1, SB *S. bayanus*, TD *T. delbrueckii*, MPSC1 sequential fermentation with *M. pulcherrima* and *S. cerevisisae* 1, MFSC1 sequential fermentation with *M. fructicola* and *S. cerevisiae*.

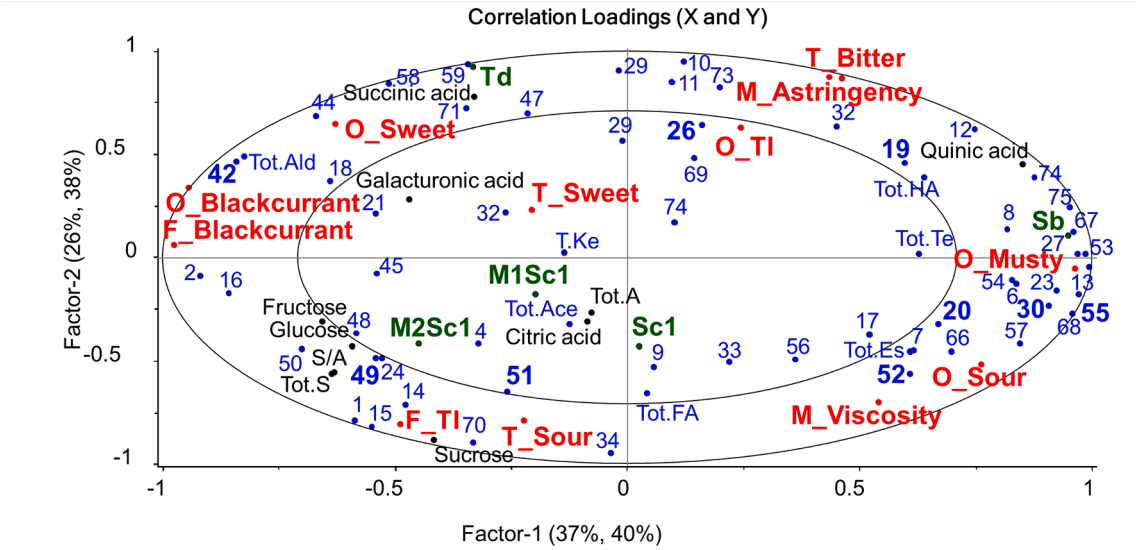


**Fig. 3.** Partial least regression of all chemical variables, their total amounts and sugar-to-acid ratio (X, n = 78, 85 % of variance, volatiles blue, sugars and organic acids black), the sensory attributes (Y, n = 12, 91 % of variance, red), and fermented beverages as dummy-variables (green). SC1 *S. cerevisiae* 1, SB *S. bayanus*, TD *T. delbrueckii*, MP *M. pulcherrima*, MF *M. fructicola*, O odour, F flavour, TI total intensity, M mouthfeel. Numbers and abbreviations of the variables refer to Table 1, and bolded numbers were the most contributing compounds observed in the GC-O analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mustiness in wines. In this study, 1-octen-3-ol was not detected because 3-(methylthio)-1-propanol was eluted at the same time. The high musty odour of the SB-fermented beverage may be the result of the fermentation temperature due to the cryotolerant nature of *S. bayanus*. Muñoz-Bernal et al. (2016) reported high concentrations of certain higher alcohols when they fermented synthetic must at 25 °C compared to 13 °C. However, many terpenes, esters, and acetates, such as D-limonene (67), ethyl decanoate (54), and phenylethyl acetate (8), are positively correlated with the SB beverage and are not observed by the panel. On the other side of the PLS regression plot, the black currant odour and flavour are located next to each other and negatively correlated with the musty odour. The sequentially fermented samples and the TD samples are positively correlated with the black currant odour and flavour. In addition, the total amounts of aldehydes, octanal (42), ethyl acetate (2), and decanoic acid (16) are positively correlated with black currant odour and flavour, and, thus, they may contribute to the black currant odour and flavour of the fermented beverages. In addition, octanal and total aldehyde contents were positively correlated with the BCJ on PC1, as shown in Fig. 1. Furthermore, the octanal SNIF value was the fifth largest in GC-O analysis indicating its potential contribution to the black currant odour.

As expected, galacturonic acid as a degradation product of pectin, and viscous mouthfeel are negatively correlated with each other. Galacturonic acid is positively correlated with the TD, MPSC1, and MFSC1 beverages on factor-2 and negatively with the SC1 and SB beverages on the factor-1. This result is in agreement with the pectinase activity of *Metschnikowia* yeasts (Belda et al., 2016) and *T. delbrueckii* (You et al., 2016). Galacturonic acid is also positively correlated with black currant odour and flavour, and sweet odour. In our previous study (Kelanne et al., 2020), we observed that a higher galacturonic acid content was positively correlated with higher anthocyanin contents in fermented black currant beverages, and, thus, more intense colour properties. All of these attributes are likely desired attributes in fermented black currant beverage. In addition to factor-1, galacturonic acid is negatively correlated with bitter taste and astringent mouthfeel, which are not desired attributes in berries (Laaksonen et al., 2016). In the sensory evaluations, the most intense sweet odour was in the TD beverage (intensity of 4.1), even though it was not significantly different from that of the other samples. In the PLS regression plot, the sweet odour was positively correlated with the TD beverage and with some chemical variables, such as nonanal (44), *E*-linalool oxide acetate (71), 3-ethoxy-1-propanol (58), and butanol (18). The content of 3-ethoxy-1-propanol was at least six-times higher in the TD beverage than in all other beverages (Table 1), which may have been the source of the sweet odour in the TD beverage.

## 4. Conclusions

This was the first time analysis of the volatile profile and sensory properties of *Saccharomyces*- and non-*Saccharomyces*-fermented black currant beverages. All fermented beverages were clearly different in their volatile compound profiles depending on the yeast used, but only small differences were observed in sensory properties. The poor distinction of fermented beverages was most likely due to the intensely sour taste, which could have masked any possible flavour differences. However, clear differences were observed between *S. bayanus* and *T. delbrueckii* fermented beverages in certain compounds, such as ethyl butanoate, furanmethanol, and ethyl 2-hydroxybutanoate. Generally, sequential fermentations and *S. cerevisiae* fermentations resulted in a higher abundance of all groups of volatile compounds compared to *T. delbrueckii* fermentations. Furthermore, *S. bayanus* fermentation differed from *S. cerevisiae* fermentation by higher contents of higher alcohols and esters. In the sequential fermentations, *Metschnikowia* yeast a greater effect on the volatile profile than *S. cerevisiae* yeast.

Non-*Saccharomyces* yeasts resulted in higher black currant odour and lower musty odour, and they exhibited characteristic pectinase activities resulting in higher contents of galacturonic acid. Although, *S. bayanus* fermentation clearly resulted in the highest total contents of esters and terpenes, it was observed as the most 'musty' fermented beverage. The musty odour may have resulted from the high total content of higher alcohols. This indicates that the chemical composition was not sufficient to reveal all differences between the fermented beverages, but the sensory evaluations demonstrated important synergy between the compounds.

The results of this study will help in the utilisation of black currants in the beverage industry and development of novel fermented black currant beverages with low ethanol content. The study demonstrated the

difference between the fermentation strategies of black currant juice indicating that sequential fermentation with *Metschnikowia* yeasts has potential in the fermentation of black currant juice. Finally, more studies are still needed concerning fermentations of black currants with non-*Saccharomyces* yeasts, such as *Pichia fermentans*, which is capable of degrading citric acid (Zhong et al., 2020), and thus may decrease the intense sourness of black currants.

## CRediT authorship contribution statement

**Niina M. Kelanne:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft. **Barbara Siegmund:** Methodology, Validation, Resources, Writing - review & editing. **Tapio Metz:** Investigation, Formal analysis. **Baoru Yang:** Resources, Writing - review & editing, Project administration. **Oskar Laaksonen:** Conceptualization, Writing - review & editing, Supervision, Project administration.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.131049.

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