

Phenolic Compound Profiles in Alcoholic Black Currant Beverages Produced by Fermentation with *Saccharomyces* and Non-*Saccharomyces* Yeasts

Niina Kelanne,* Baoru Yang, Laura Liljenbäck, and Oskar Laaksonen



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ABSTRACT: Alcoholic beverages with low ethanol contents were produced by fermenting black currant juice with *Saccharomyces* and non-*Saccharomyces* yeasts without added sugar. The effects of different yeasts on the phenolic compounds (anthocyanins, hydroxycinnamic acids, flavonols, and flavan-3-ols) and other selected constituents (the ethanol content, residual sugars, organic acids, and color) of the black currants were assessed. Single yeast-fermented beverages had higher ethanol contents (3.84–4.47%, v/v) than those produced by sequential fermentation. In general, the fermentation of black currant juice increased the contents of organic acids and flavonols, whereas anthocyanin contents decreased. All of the fermentations decreased the contents of glycosylated nitrile-containing hydroxycinnamic acids, resulting in higher contents of the corresponding aglycons. Fermentation with *Saccharomyces bayanus* resulted in lower anthocyanin and organic acid contents compared to the other yeasts. Sequential fermentations with *Saccharomyces cerevisiae* and *Metschnikowia pulcherrima* led to the highest total hydroxycinnamic acids and anthocyanins among all of the fermentations.

KEYWORDS: black currant, fermentation, *Saccharomyces* yeast, non-*Saccharomyces* yeast, phenolic compounds

1. INTRODUCTION

Black currant (*Ribes nigrum*) is the third most cultivated berry crop in Finland and the second largest berry crop in Europe. The phenolic composition of black currants has been widely studied and varies according to the cultivar, growth location, and weather conditions.^{1,2} The rich phenolic contents of the berries affect their sensory properties, resulting in a bitter taste and more astringency.³ The phenolic compounds in black currants also have potential beneficial effects on human health.^{4,5} Anthocyanins are the primary group of phenolic compounds in black currants, followed by flavonols, flavan-3-ols, and phenolic acids.^{6,7}

Wine yeasts have a comprehensive effect on the sensory quality of final wine products, and therefore, selecting the yeast is an important task.^{8–10} *Saccharomyces cerevisiae* and *Saccharomyces bayanus* are the most frequently used wine yeast species as a result of their high ethanol tolerance and good fermentation performance in matrices with high sugar contents and low pH values. Non-traditional wine yeasts, such as *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, and *Metschnikowia fructicola*, have become increasingly popular in research on fruit wine fermentation in recent years, and these yeasts are commercially available for fine production at an industrial scale as well. Non-*Saccharomyces* wine yeasts are often used to improve the comprehensive quality parameters, such as increasing the formation and/or release of aromatic volatiles,¹¹ and, thus, possibly improve the sensory properties,⁸ reduce the alcohol content,⁹ and improve the stability of the anthocyanin pigments.¹⁰ As a result of their relatively low fermentation performance and ethanol tolerance,

non-*Saccharomyces* yeasts are primarily used in mixed or sequential fermentations with *S. cerevisiae*.

Yeast fermentation is known to affect the composition of anthocyanins and pyranoanthocyanins, a group of anthocyanins primarily present in alcoholic beverages. They are anthocyanin derivatives formed by condensation between anthocyanins and yeast metabolites, such as pyruvic acid, acetaldehyde, and vinyl-phenols. The color of pyranoanthocyanins is usually reddish brown or reddish orange, and their typical maximal absorption wavelength is typically between 495 and 515 nm,¹² lower than those of the corresponding monomeric anthocyanins. Pyranoanthocyanins are formed during the wine fermentation mostly when 20–85% of the glucose is consumed.¹³ However, their formation is a slow process that takes more time than sugar fermentation.¹¹ The formation of some pyranoanthocyanins requires the presence of oxidants to be completed.¹³

During fermentation, yeasts produce intra- and extracellular enzymes, such as pectinases, esterases, proteases, and glycosidases, which have effects on the technological and sensory properties of the final wine products.^{14,15} The production rate of the enzymes depends upon the yeast species and strain.^{14–16} Pectinases degrade pectin, which results in improved clarification and filtration

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as well as the release of phenolic compounds, such as anthocyanins and flavonols, in addition to flavor compounds.¹⁶ β -Glucosidase is a common glycosidase produced by the wine yeasts. It can increase or decrease the final quality of a wine by releasing glycoside moieties from terpenoids (increasing the aroma complexity¹⁷) and anthocyanins (decreasing the color intensity¹⁵).

The effects of processing, such as heat, enzymatic treatment,^{3,18,19} and storage,²⁰ on phenolic compounds and, thus, the sensory properties of black currants have been widely studied. However, there are only a few studies focused on the effects of yeast fermentation on the chemical composition of black currant beverages.^{21–27} Moreover, no study reporting on comparisons of the impacts of different yeasts on the chemical composition of fermented black currant were found. In the present study, we aimed to assess comprehensive effects of pure fermentation with single yeast strains of *S. cerevisiae*, *S. bayanus*, or *T. delbrueckii* yeasts as well as sequential fermentations of *M. pulcherrima* and *M. fructicola* with *S. cerevisiae* on the chemical composition of black currant juice. Special focus was directed toward the effects on various non-volatile constituents, such as phenolic compounds, sugars, and organic acids, as well as ethanol, which are known to impact the sensory properties of beverages strongly. Black currant pulp containing a high amount of pectin was used in this study because its flavor properties resemble those of the original black currants more than those of the enzymatically treated black currants.²⁸ Additionally, sugars were not added to the juice before or during fermentation because our goal was to produce beverages with a low ethanol concentration instead of fruit wines.

2. MATERIALS AND METHODS

2.1. Yeasts and Standard Compounds. The two *S. cerevisiae* strains W15 and ICV-K1 (Sc1 and Sc2, Lalvin, Montreal, Quebec, Canada), *T. delbrueckii* Biodiva (Td, Level, Edwardstown, Australia), *M. pulcherrima* Flavia (M1, Level, Edwardstown, Australia), and *M. fructicola* IOC Gaia (M2, Edwardstown, Australia) were kindly provided by Lallemand, Inc. (Montreal, Quebec, Canada), and *S. bayanus* (Sb, Condessa, Viinitalo Melkko, Ltd., Lahti, Finland) was purchased from a local wine equipment store in Turku, Finland.

Quercetin-3-O-glucoside, cyanidin-3-O-glucoside, and (+)-catechin were purchased from Extrasynthese (Genay, France). *p*-Coumaric acid, chlorogenic acid, xylitol, tartaric acid, quinic acid, fumaric acid, shikimic acid, and galacturonic acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Citric acid and sucrose were purchased from J.T. Baker Chemicals (Leuven, Belgium). *n*-Butanol was purchased from Riedel-de Haën (Morris Plains, NJ, U.S.A.), and ethanol was purchased from Altia (Helsinki, Finland).

2.2. Preparation of Black Currant Juice and Fermented Beverages. Frozen Finnish black currants (Pakkasmarja, Ltd., Suonenjoki, Finland) were purchased from a local supermarket. All of the black currants used here were from the same production batch. They were stored at $-20\text{ }^{\circ}\text{C}$ until further use. Before the juice pressing, the black currants were thawed in the microwave oven for 1.5 min at 350 W, mixed, and thawed again for 1.5 min at 350 W. Thawed berries were cold-pressed with a vertical tincture press (HP 2 H, Hafco, Fischer Maschinenfabrik, Neuss, Germany) at a pressure of 140 kg/cm^3 . The black currant juice was pasteurized in 50 mL glass bottles by immersing the bottles into boiling water. The temperature changes in the juice were followed with a thermometer (TM-947SD, Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan) until it reached $97\text{ }^{\circ}\text{C}$, kept at $97\text{ }^{\circ}\text{C}$ for 30 s, and then immediately transferred to ice to cool to $25\text{ }^{\circ}\text{C}$. For each fermentation, 100 mL of pasteurized juice was measured and transferred to a glass bottle.

Before inoculation of the black currant juice, all of the yeasts were reactivated with water rehydrant solution (Go-Ferm, Lallemand, Inc., Montreal, Quebec, Canada) to shorten the lag phase of the

fermentation. The optimal temperature and time were used to reactivate each yeast, which were as follows: *S. cerevisiae* 1, $35\text{--}40\text{ }^{\circ}\text{C}$; *S. cerevisiae* 2, $40\text{ }^{\circ}\text{C}$; *S. bayanus*, $30\text{--}35\text{ }^{\circ}\text{C}$; *T. delbrueckii*, $30\text{ }^{\circ}\text{C}$; *M. pulcherrima*, $30\text{ }^{\circ}\text{C}$; and *M. fructicola*, $20\text{--}30\text{ }^{\circ}\text{C}$. The minimum reactivation time was 20 min for every yeast but less than 45 min. The inoculation was performed so that the inoculation amount was 0.25 g/L of active dried yeast, which corresponds to 1.4×10^8 colony-forming units (CFU)/mL of *S. cerevisiae* 1, 3.6×10^9 CFU/mL of *S. cerevisiae* 2, 9.7×10^7 CFU/mL of *S. bayanus*, 4.7×10^8 CFU/mL of *T. delbrueckii*, 7.3×10^8 CFU/mL of *M. pulcherrima*, and 1.2×10^8 CFU/mL of *M. fructicola*. The cell populations of each yeast were determined by plate counting on YPD agar (1% yeast extract, 2% peptone, 2% dextrose, and 2% agar). The plates were incubated at $35\text{ }^{\circ}\text{C}$ for 48–72 h before counting. The fermentation was performed at $21\text{ }^{\circ}\text{C}$ in the dark. For sequential fermentations, the juice was pre-fermented with *Metschnikowia* yeasts for 24 h, and after that, *S. cerevisiae* yeast was added to continue the fermentation, as described above. All of the fermentations were performed in triplicate.

The fermentations were monitored by measuring the Brix (Hanna Instruments, Woonsocket, RI, U.S.A.) regularly and were stopped by adding yeast killer (potassium sulfate/potassium sorbate, 1:1, Jässtopp D, Viinitalo Melkko, Lahti, Finland) when the Brix had remained constant for 24 h. The beverages were centrifuged at 1500g for 10 min, and the supernatant was collected and stored in the freezer at $-80\text{ }^{\circ}\text{C}$.

2.3. Extraction of Phenolic Acids and Flavonol Glycosides. To extract the non-anthocyanin phenolic compounds, 2 g of each sample was weighed in an extraction tube and 5 mL of ethyl acetate was added. Thereafter, the extraction tube was shaken vigorously for 90 s, followed by centrifugation for 5 min at 1500g . The supernatant was collected in an evaporation flask. The extraction was repeated 4 times, and the extracts were combined. Ethyl acetate was evaporated to dryness at $35\text{ }^{\circ}\text{C}$ under a vacuum with a rotary evaporator (Heidolph Instruments, Schwabach, Germany), and the extract was reconstituted with 1 mL of methanol and filtered through a $0.22\text{ }\mu\text{m}$ polytetrafluoroethylene (PTFE) filter. The extractions were performed in triplicate.

2.4. Determination of Phenolic Acids, Flavonols, and Anthocyanins. The phenolic acids, flavonol glycosides, flavan-3-ols, methyl citrates, and anthocyanins were determined using an ultra-high-performance liquid chromatography (UHPLC) instrument (Nexera 30 Series, Shimadzu Corp., Kyoto, Japan) coupled with a SPD-M20A diode array detector (DAD), as described previously by Mäkilä et al.,²⁰ with slight modifications. The mobile phases were water (A) and acetonitrile (B), both containing formic acid (0.1%, v/v). The phenolic acids, flavonols, flavan-3-ols, and methyl citrates were separated with the following mobile phase B gradient: 0–15 min, 2–18%; 15–20 min, 18%; 20–30 min, 18–20%; 30–35, 20–60%; and 40–45 min, 60–2%. The flow rate of the mobile phase was 1 mL/min. The separation of compounds was performed with an AerisPeptide column (XB-C18, 150 mm \times 4.6 mm \times 3.6 μm , Phenomenex, Torrance, CA, U.S.A.). The oven temperature was set to $25\text{ }^{\circ}\text{C}$. Flavan-3-ols and methyl citrates were recorded at 280 nm; phenolic acids were recorded at 310 and 320 nm; and flavonol glycosides were recorded at 360 nm. (+)-Catechin, *p*-coumaric acid, chlorogenic acid, and quercetin-3-O-glucoside were used to quantify the compounds at 280, 310, 320, and 360 nm, respectively. Five-point calibration curves were constructed using reference compounds over a concentration range of 0.006–0.40 g/L.

Before the anthocyanin analysis, 500 μL of black currant juice or fermented beverage was diluted with 500 μL of acidified methanol (using concentrated HCl at 1%, v/v) and filtered through a $0.22\text{ }\mu\text{m}$ PTFE filter. The mobile phases were water (A) and acetonitrile (B), both containing formic acid (5%, v/v). The following gradient was used for the mobile phase B: 0–10 min, 5–10%; 10–15 min, 10–15%; 15–20 min, 15–40%; 20–30 min, 40–90%; and 30–35 min, 90–5%. The flow rate was 1 mL/min. The separation of the compounds was performed with the same column as mentioned previously. The oven temperature was $36\text{ }^{\circ}\text{C}$, and the peaks were recorded at 520 nm. Cyanidin-3-O-glucoside was used to construct a five-point calibration curve over a concentration range of 0.021–0.34 g/L to quantify the anthocyanins.

2.5. Qualitative Analysis of Phenolic Acids, Flavonols, and Anthocyanins. The phenolic acids, flavonol glycosides, and anthocyanins were identified with an Elute UHPLC–DAD instrument coupled with an Impact II ESI–QTOF system (Bruker Daltonik GmbH, Bremen, Germany). The system was controlled with Compass HyStar software (Bruker Daltonik GmbH, Bremen, Germany). The same mobile phases A and B were used as those described previously. In the analysis of flavan-3-ols, phenolic acids, and flavonols, the following mobile phase B gradient was used: 0–9.4 min, 2–18%; 9.4–11.80 min, 18%; 11.80–14.20 min, 18–20%; 14.20–16.50 min, 20–60%; 16.50–18.90 min, 60–2%; and 18.9–21.20 min, 2%. The flow rate was 0.5 mL/min. The compounds were separated with a bioZen column (XB-C18, 150 mm × 2.1 mm × 1.7 μm, Phenomenex, Torrance, CA, U.S.A.). The oven temperature was set to 35 °C, and the peaks were recorded at 310, 320, and 360 nm. The mass spectrometer was operated in positive- and negative-ion modes. The capillary voltage was set to 4.5 kV; the nebulizer pressure was set to 4.8 bar; the dry gas flow was set to 12 L/h; and the dry temperature was set to 350 °C. The ions were scanned over a range of m/z 20–1000.

The chromatographic conditions and column used for the qualitative anthocyanin analysis were the same as those used previously for quantitative analysis. A flow of 0.140 μL/min was directed to the mass spectrometer operated in positive- and negative-ion modes. The capillary voltage was 4.5 kV; the nebulizer pressure was 1.4 bar; the dry gas flow was 9.0 L/h; and the dry temperature was 230 °C. The ions were scanned over a range of m/z 20–1000.

2.6. Determination of Sugars and Organic Acids. The sugars and organic acids were analyzed with gas chromatography (GC, GC-2010Plus, Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector (FID) as trimethylsilyl (TMS) derivatives as described before in our previous study.²⁹ The compounds were quantified using the internal standards: xylitol for sugars and tartaric acid for acids at a concentration of 5 g/L. External standards (quinic, fumaric, shikimic, citric, and galacturonic acids, all at 5 g/L) were analyzed to identify and calculate the correction cofactors.

2.7. Ethanol Determination in Fermented Beverages. The ethanol was determined with GC (GC-2010Plus, Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector (FID) as described before by Liu et al.³⁰ Before the analysis, the samples were filtered with 0.45 μm RC filters. 2-Butanol was added as an internal standard for ethanol quantification.

2.8. Determination of Color Properties. The color properties were determined from undiluted samples by spectrophotometric measurements (Evolution UV–vis 300, Thermo Fisher Scientific, Wilmington, DE, U.S.A.) using a plastic cell with a 1 cm optical pathway. The absorbances were recorded at 420, 520, and 620 nm. According to Liu et al.,³⁰ percentages of yellow (% yellow), red (% red), and blue (% blue) and the color intensity (CI) and color tonality (CT) were calculated as follow:

$$\text{percentage of color} = \frac{A_{420/520/620}}{CI}$$

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

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

2.9. Statistical Analysis. SPSS 25.0.0.1. (IBM SPSS Statistics, Inc., Chicago, IL, U.S.A.) used a one-way analysis of variance (ANOVA) with Tukey's test and an independent sample t test. The one-way ANOVA was used to determine the statistical difference between different yeast fermentations, and the independent sample t test was used to determine the differences between the black currant juice and the average fermented beverage. Unscrambler X (version 11, CAMO, Inc., Oslo, Norway) was used to perform principal component analysis (PCA1). PCA1 was constructed using one black currant juice sample, three biological replicates of seven fermented beverage samples, and 57 chemical variables. The samples were grouped by juice and the yeast species into seven groups. PCA2 was constructed using three biological replicates of seven fermented beverage samples,

and there were 57 chemical variables. The samples were grouped by the yeast species.

3. RESULTS AND DISCUSSION

3.1. Fermentation Kinetics. The fermentation times between the single yeast and sequential fermentations were notably different. Sequential fermentations with *M. pulcherrima* (M1) or *M. fructicola* (M2) and the *S. cerevisiae* strains (Sc1 and Sc2) lasted only 6 days, whereas the single yeast fermentations took 8–11 days to complete. The fermentation kinetics measured as changes in Brix values are presented in Figure 1.

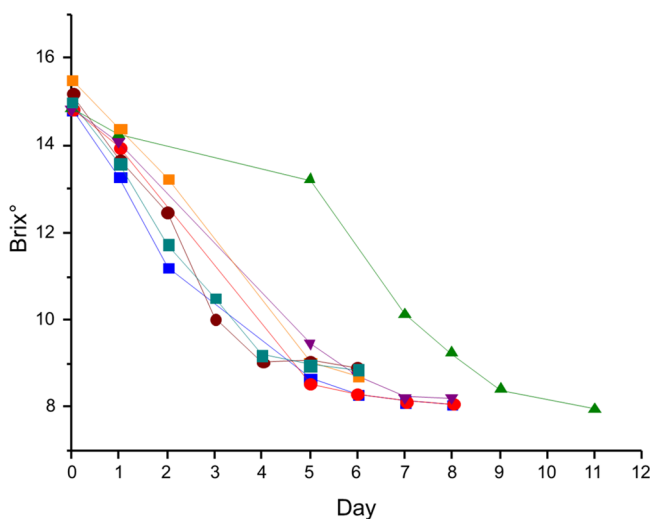


Figure 1. Decrease in Brix values during the fermentation: (blue ■) *S. cerevisiae* 1, (red ●) *S. cerevisiae* 2, (green ▲) *S. bayanus*, (purple ▼) *T. delbrueckii*, (orange ■) *M. pulcherrima* + *S. cerevisiae* 1, (cyan ■) *M. fructicola* + *S. cerevisiae* 1, and (maroon ●) *M. pulcherrima* + *S. cerevisiae* 2.

The Brix values of the M1Sc2 and M2Sc1 fermentations also decreased less than in other fermentations according to the initial Brix of the juice. The single yeast fermentation with *S. bayanus* (Sb) had the longest lag phase, and its fermentation lasted the longest. Similar low fermentation rates for *S. bayanus* have been reported before.³¹

The pH was measured before and after each fermentation (Table 1). The initial pH of the black currant juice was 2.96, and after fermentation the values varied between 2.93 (M2Sc1) and 3.07 (Sb). The *S. bayanus* and *T. delbrueckii* (Td) fermentations increased the pH significantly compared to the black currant juice. The increase in the pH cannot be explained by the compounds determined in this study, but, for example, changes in the pectin concentration could have an effect on the pH.³²

3.2. Ethanol Concentration. The highest ethanol concentration was observed in Sc2 (4.47%, v/v), and the lowest ethanol concentration was in M1Sc2 (3.46%, v/v; Table 1). The ethanol concentration in the Sc2 beverage was significantly higher than those in the beverages from all of the sequential fermentations (3.46–3.79%). A lower ethanol content of sequential fermentations is possibly caused by the lower ethanol production performance of *Metschnikowia* yeasts. Contreras et al.⁹ reported significantly lower levels of ethanol from sequential fermentation with *S. cerevisiae* and five different *M. pulcherrima* yeast strains than fermentation with *S. cerevisiae* alone. However, Escribano-Viana et al.³² did not observe almost any difference in the ethanol concentration with sequential fermentations compared

Table 1. Physicochemical Parameters of Black Currant Juice and Fermented Beverages^a

number	juice	Sc1	Sc2	Sb	Td	MISc1	MISc2	M2Sc1	averaged beverage ^b
pH	2.96 ± 0.05 ab	2.95 ± 0.00 a	2.99 ± 0.01 abc	3.07 ± 0.02 c	3.06 ± 0.01 c	3.00 ± 0.04 abc	2.93 ± 0.01 ab	3.03 ± 0.05 bc	3.00 ± 0.05
ethanol (% v/v)	nd	3.84 ± 0.3 ab	4.47 ± 0.4 c	4.17 ± 0.4 bc	4.07 ± 0.5 bc	3.69 ± 0.4 ab	3.46 ± 0.2 a	3.79 ± 0.4 ab	3.9 ± 0.5 ^c
				Organic Acids (g/L)					
1A	succinic acid	0.7 ± 0.06 b	0.7 ± 0.02 b	0.9 ± 0.08 c	0.8 ± 0.03 b	0.5 ± 0.02 a	0.5 ± 0.2 a	0.5 ± 0.03 a	0.6 ± 0.2
2A	malic acid	2.8 ± 0.1	2.8 ± 0.1	2.6 ± 0.2	2.8 ± 0.06	2.8 ± 0.1	2.8 ± 0.1	2.6 ± 0.2	2.7 ± 0.4
3A	shikimic acid	0.4 ± 0.03 cd	0.4 ± 0.01 abc	0.4 ± 0.03 ab	0.3 ± 0.01 a	0.4 ± 0.02 d	0.4 ± 0.02 ab	0.4 ± 0.03 bc	0.4 ± 0.05
4A	citric acid	31.8 ± 3.4 ab	32.1 ± 2.0 ab	31.1 ± 3.0 a	33.2 ± 2.1 ab	35.1 ± 2.5 b	32.2 ± 2.3 ab	30.3 ± 3.1 a	31.9 ± 4.5
5A	quinic acid	0.5 ± 0.03 c	0.2 ± 0.01 ab	0.2 ± 0.02 a	0.2 ± 0.01 ab	0.3 ± 0.01 b	0.2 ± 0.01 a	0.2 ± 0.02 ab	0.2 ± 0.03
6A	ascorbic acid	0.9 ± 0.1 ab	0.9 ± 0.06 ab	0.8 ± 0.1 a	0.9 ± 0.03 abc	1.0 ± 0.07 c	0.9 ± 0.05 ab	1.0 ± 0.08 bc	0.9 ± 0.1
7A	galacturonic acid	0.2 ± 0.03 b	0.2 ± 0.02 b	0.2 ± 0.03 a	0.2 ± 0.08 b	0.3 ± 0.1 c	0.3 ± 0.03 c	0.3 ± 0.04 bc	0.2 ± 0.1
	total	36.6 ± 0.4 ab	37.3 ± 2 ab	36.2 ± 3 ab	38.4 ± 2 ab	40.5 ± 3 b	37.2 ± 3 ab	35.1 ± 4 a	37 ± 6
				Sugars (g/L)					
1S	fructose	0.1 ± 0.05 a	0.1 ± 0.02 a	1.9 ± 0.1 b	0.2 ± 0.1 a	nd	nd	nd	0.3 ± 0.1
2S	glucose	0.08 ± 0.08 a	0.2 ± 0.08 ab	1.0 ± 0.08 c	0.2 ± 0.08 b	nd	nd	nd	0.02 ± 0.05
3S	sucrose	2.8 ± 0.5 c	0.4 ± 0.3 a	0.1 ± 0.08 a	1.1 ± 0.1b	3.9 ± 0.3 d	3.9 ± 0.3 d	3.5 ± 0.5 d	0.2 ± 0.2
	total (g/L)	3.0 ± 0.5 cd	0.6 ± 0.4 a	2.3 ± 1.4 bc	1.5 ± 0.2 ab	3.9 ± 0.3 d	3.9 ± 0.3 d	3.5 ± 0.5 d	0.3 ± 0.1
				Color Analysis					
	yellow (%)	35.0 ± 0.1 bc	34.2 ± 0.2 a	36.2 ± 0.2 d	35.5 ± 0.7 cd	34.3 ± 0.6 a	34.2 ± 0.4 a	34.2 ± 0.4 a	na
	red (%)	34.3 ± 0.1 a	34.0 ± 0.1 a	35.5 ± 0.2 c	34.9 ± 0.4 b	33.6 ± 0.03 a	33.6 ± 0.04 a	33.6 ± 0.1 a	na
	blue (%)	30.8 ± 0.1 c	31.8 ± 0.1 cd	28.4 ± 0.2 a	29.6 ± 1.0 b	32.1 ± 0.6 cd	32.2 ± 0.3 cd	32.2 ± 0.4 cd	na
	color intensity	7.1 ± 0.1 abc	7.3 ± 0.1 cd	6.9 ± 0.1 a	7.0 ± 0.2 ab	7.2 ± 0.2 bcd	7.3 ± 0.1 bcd	7.3 ± 0.1 bcd	na
	color tonality	1.02 ± 0.01	1.01 ± 0.01	1.02 ± 0.01	1.02 ± 0.01	1.02 ± 0.02	1.02 ± 0.01	1.02 ± 0.01	na

^aA, organic acid; S, sugar; nd, not detected; na, not calculated; Sc1, *S. cerevisiae* 1; Sc2, *S. cerevisiae* 2; Sb, *S. bayanus*; Td, *T. delbrueckii*; MISc1, *M. pulcherrima* and *S. cerevisiae* 2; and M2Sc1, *M. fructicola* and *S. cerevisiae* 1. The results are expressed as the average of three biological replicates ± standard deviation. The same letters in each row indicate that there is no significant ($p > 0.05$) difference between samples. ^bThe averaged beverage was calculated from all of the biological replicates of all of the fermentations. ^cStatistically significant differences between black currant juice and the average beverage ($p < 0.05$).

to a conventional single yeast fermentation with *S. cerevisiae*. Our results indicate that Sc2 has a better ethanol production performance than Sc1 in fermenting black currant juice. In addition to the difference between the yeast strains, the ethanol content is often affected to a great extent when another yeast is introduced into the fermentation matrix. Typically, *T. delbrueckii* is used to reduce the ethanol content in grape wines,³³ whereas our study demonstrated that black currant juice fermented with Td had a higher ethanol content (4.07%, v/v) than the Sc1-fermented sample (3.84%, v/v). *T. delbrueckii* is reportedly highly resistant to stressful environments, such as low pH and temperature.³⁴ This resistance may explain the similar or better fermentation performance of *T. delbrueckii* in black currant juice in comparison to some *S. cerevisiae* yeast strains. In addition, *T. delbrueckii* can be used alone to ferment black currant juice.

3.3. Identification of Phenolic Compounds. A total of 27 phenolic acids, 12 flavonols, 3 proanthocyanidins, and 16 anthocyanins were identified or tentatively identified (Table 2) in the black currant juice and fermented beverages by through comparisons of the retention times, ultraviolet–visible (UV–vis) spectra, and mass spectra to the reference compounds and the results reported in the literature.^{3,20,35–37}

In the phenolic acid group, derivatives of caffeic, *p*-coumaric, and ferulic acids were detected in addition to free caffeic acid and *p*-coumaric acid. Four phenolic acids containing a nitrile group were also detected in the black currant juice: (*E*)-caffeoyloxymethyleneglucosyloxybutenenitrile, (*E*)-coumaroyloxymethyleneglucosyloxybutenenitrile, (*Z*)-coumaroyloxymethyleneglucosyloxybutenenitrile, and (*E*)-feruloyloxymethyleneglucosyloxybutenenitrile (compounds 10–13 in Table 2), which have been identified before by Lu et al. and Mäkilä et al.^{20,38} In comparison to the black currant juice, two new phenolic acid peaks were detected only in the fermented samples, namely, (*E*)-coumaroyloxymethylenoxybutenenitrile and (*Z*)-coumaroyloxymethylenoxybutenenitrile (compounds 14 and 15 in Table 2). They were tentatively identified on the basis of the mass spectra as aglycones of compounds 11 and 12, respectively. The odd-numbered nominal mass [*M*] of *m/z* 259 indicated the presence of odd-numbered nitrogen atoms in the molecules. The fragmentation pattern under both ionization modes (*m/z*+ 96, 114, 147, and 242 [*M* + H – H₂O]; *m/z*– 117 and 163 [*M* – H – coumaric acid]) were similar to those of compounds 11 and 12. These compounds are most likely formed through the cleavage of glucose by yeast glucosidases.

Flavonols were identified as glycosides of myricetin, quercetin, and kaempferol, and anthocyanins were identified as glycosides of delphinidin, cyanidin, and peonidin. Two pyranoanthocyanins, cyanidin-3-*O*-rutinoside-pyruvic acid and cyanidin-3-*O*-rutinoside-acetaldehyde (compounds 39 and 40 in Table 2), were detected and tentatively identified in fermented beverages with a quadrupole time-of-flight (QTOF) mass spectrometer. The parent ion masses were [*M* + H]⁺ 663 and 619, respectively. The daughter ions of the peaks were both in *m/z*+ 595, indicating that both compounds were cyanidin-3-*O*-rutinoside derivatives. Compound 39 was tentatively identified as cyanidin-3-*O*-rutinoside-pyruvic acid ([*M* + H + pyruvic acid]⁺ 595 + 68), and compound 40 was cyanidin-3-*O*-rutinoside-acetaldehyde ([*M* + H + acetaldehyde]⁺ 595 + 24). Pyranoanthocyanins were detected in the trace amounts in all fermented beverages but were absent from the black currant juice. The formation of a low amount of pyranoanthocyanins during black currant fermentation could be attributed to low concentrations of nutrients, such as sugars and nitrogen, which limited the formation of pyruvic

acid and acetaldehyde during the early stage of fermentation. The sugar content of the grape musts ranges between 150 and 250 g/L, whereas in the black currant juice used in this study, it was only 78 g/L. In a nutrient-rich environment, pyruvic acid is produced in excess and excreted from yeast cells into the fermentation medium, which leads to a high formation rate of pyranoanthocyanins in wines. Additionally, the pH and temperature affect the formation rate of pyranoanthocyanins.³⁹ The pH of grapes must can be as high as 3.6,⁴⁰ whereas the black currant juice pH is typically below 3 (in this study, it was 2.96).⁴¹

3.4. Comparison between Chemical Compositions of Black Currant Juice and Fermented Beverages. The independent sample *t* test was used to compare the phenolic compositions of black currant juice and the averaged value of all fermented black currant beverages (Table 3). Generally, the phenolic profiles differed between the fermented beverages and the black currant juice.

Seven organic acids and three sugars were detected in the juice and fermented beverages (Table 1). Not surprisingly, the contents of all of the sugars were significantly lower in the fermented beverages than in the black currant juice. Interestingly, the total organic acids differed less than 1% between the juice and average beverage as a result of the fact that citric acid as the primary acid remained unaffected. Citric acid accounted for up to 87% of the total organic acid contents. Methyl esters of citric acid have been detected before in black currants²⁰ and were also detected in the juice and fermented beverages in our study. The average concentrations of citric acid methyl esters were statistically significantly higher in fermented black currant beverages (73 mg/L) than the corresponding values in the black currant juice (67 mg/L). In contrast, the fermentations primarily decreased the concentrations of malic, shikimic, and quinic acids. Succinic and galacturonic acids were not present in the black currant juice but appeared in the all of the fermentations (Table 3). Yeasts produce succinic acid during alcohol fermentation through a few different metabolic pathways,⁴² and galacturonic acid is formed as a degradation product of pectin by pectinases.

Fermentation decreased the total concentration of hydroxycinnamic acids (by 6.7%) and the total content of anthocyanins (by 28.4%) and increased the total concentration amounts of flavonols (11.7%) and flavan-3-ols (8.8%). The contents of all of the hydroxycinnamic acids containing a nitrile moiety decreased significantly during the fermentations compared to the black currant juice. In addition, the contents of *Z*-*p*-coumaroylglucose, ferulic acid hexoside, and *p*-coumaric acid were significantly higher in the fermented beverages than in the juice. Czyżowska and Pogorzelski²² reported that the hydroxycinnamic acid contents decreased by approximately half during fermentation. In general, all monomeric anthocyanin contents decreased during the fermentations. The most highly affected anthocyanins were the glucosides of cyanidin, delphinidin, and cyanidin 3-*O*-(6-*p*-coumaroyl) glucoside. Their contents decreased by 40.8, 37.5, and 34.7%, respectively. Simultaneously, the concentrations of delphinidin and cyanidin aglycones increased during the fermentations. Anthocyanins have an important role in consumer acceptance of food products because their color is one of the first things consumers can perceive in foods. Czyżowska and Pogorzelski²¹ reported a higher loss of anthocyanins (96%) after a wine yeast fermentation of black currant juice treated with pectinase compared to our results.

A PCA model constructed with all of the samples (*n* = 21; biological replicates shown separately) and all of the chemical

Table 2. Identification of Hydroxycinnamic Acid Derivatives, Flavonols, Flavan-3-ols, and Anthocyanins in Fermented Black Currant Beverages

compound number	tentative identification ^a	λ_{\max} (nm)	MS (<i>m/z</i>) ⁺		MS ²		MS (<i>m/z</i>) ⁻		MS ²		exact mass (molecule)	M (error, ppm)	molecular formula	identification	ref
			[M + H] ⁺ mass/calculated mass	[M + H] ⁺ (error ppm)	[M + H] ⁺ (error ppm)	[M + H] ⁺	[M - H] ⁻ mass/calculated mass	[M - H] ⁻ (error ppm)	[M - H] ⁺	measurement/calculated					
1	citric acid methyl ester	195, 294	nd	nd	nd	205.0343/205.0354	5.0	191.0176, 111.0075, 143.0332	206.0416/206.0427	-5.3	C ₇ H ₁₀ O ₇	UV, QTOF	20		
Hydroxycinnamic Acids															
2	4-O-caffeoylglucoside	215, 328	nd	nd	nd	341.0846/341.0878	9.3	161.0230, 133.0280	342.0919/342.0951	-9.4	C ₁₅ H ₁₈ O ₉	UV, QTOF	20		
3	<i>p</i> -coumaric acid O-glucoside	208, 294	nd	nd	nd	325.0900/325.0929	9	163.0386, 119.0486	326.0973/326.1002	-8.9	C ₁₅ H ₁₈ O ₈	UV, QTOF	20		
4	(<i>E</i>)-caffeoylglucose	216, 328	nd	nd	nd	341.0845/341.0819	7.6	161.0226, 133.0278, 683.1716	342.0918/342.0892	7.6	C ₁₅ H ₁₈ O ₉	UV, QTOF	20		
5	<i>p</i> -coumaroylquinic acid	208, 311	339.1075/339.1074	-0.1	147.0446, 119.0486	337.0890/337.0870	-5.8	163.0382	338.0963/338.0943	5.9	C ₁₆ H ₁₆ O ₈	UV, QTOF	20		
6	(<i>Z</i>)- <i>p</i> -coumaric acid O-glucoside	210, 311	nd	nd	nd	325.0924/325.0929	1.5	145.0291	326.0997/326.1002	-1.5	C ₁₅ H ₁₈ O ₉	UV, QTOF	20		
7	caffeic acid	217, 323	181.0512/181.0495	-9.3	163.0405	179.0339/179.0350	6	161.0229, 339.0679	180.0412/180.0423	-6.1	C ₉ H ₈ O ₄	UV, QTOF	7,20		
8	ferulic acid hexoside	214, 327	nd	nd	nd	355.0988	nd	193.0481, 175.0378	356.1061	nd	nd	UV, QTOF	7,20		
9	<i>p</i> -coumaric acid	207, 309	nd	nd	nd	nd	nd	nd	nd	nd	nd	STD, HPLC	7		
10	2-(<i>E</i>)-caffeoyloxymethylene-4- β -D-glucopyranosyloxy-2-(<i>Z</i>)-butenenitrile [(<i>E</i>)-caffeoyloxymethylene glucosyloxybutenenitrile]	213, 330	nd	nd	nd	436.1191/436.1190	nd	nd	437.1264/437.1263	0.2	nd	UV, QTOF	20		
11	2-(<i>E</i>)- <i>p</i> -coumaroyloxymethylene-4- β -D-glucopyranosyloxy-2-(<i>Z</i>)-butenenitrile [(<i>E</i>)-coumaroyloxymethyleneglucosyloxybutenenitrile]	210, 313	422.1445/422.1446	0.1	114.0549, 147.0436	420.1257/420.1255	-0.4	163.0385, 119.0492	421.133/421.1328	0.5	C ₂₀ H ₂₃ NO ₉	UV, QTOF	20		
12	2-(<i>Z</i>)- <i>p</i> -coumaroyloxymethylene-4- β -D-glucopyranosyloxy-2-(<i>Z</i>)-butenenitrile [(<i>Z</i>)-coumaroyloxymethyleneglucosyloxybutenenitrile]	216, 310	422.144/422.1446	0.5	114.0548	420.1256/420.1300	nd	163.0389	421.1329/421.1373	-10.4	C ₂₀ H ₂₃ NO ₉	UV, QTOF	20		
13	2-(<i>E</i>)-feruloyloxymethylene-4- β -D-glucopyranosyloxy-2-(<i>Z</i>)-butenenitrile [(<i>E</i>)-feruloyloxymethylene glucosyloxybutenenitrile]	218, 327	452.1543/452.1551	0.8	114.0539, 177.0542	450.1355/450.1397	nd	193.0485	451.1428/451.1470	-9.3	C ₂₁ H ₂₃ NO ₁₀	UV, QTOF	20		
14	2-(<i>E</i>)- <i>p</i> -coumaroyloxymethylenesylloxy-2-(<i>Z</i>)-butenenitrile [(<i>E</i>)-coumaroyloxymethylenesylloxybutenenitrile]	217, 313	nd	nd	nd	258.0756/258.0771	nd	163.0390, 184.0755, 214.0859	259.0829/259.0844	-5.8	C ₁₄ H ₁₃ NO ₄	UV, QTOF	20		

Table 2. continued

compound number	tentative identification ^a	MS (m/z)+		MS ²		MS (m/z)−		MS ²	exact mass (molecule)	M (error, ppm)	molecular formula	identification	ref
		[M + H] ⁺ exact mass/calculated mass	[M + H] ⁺ (error ppm)	[M + H] ⁺	[M − H] [−] exact mass/calculated mass	[M − H] [−] (error, ppm)	[M − H] ⁺						
15	2-(Z)-p-coumaroyloxymethylensylloxy-2-(Z)-butenenitrile [(Z)-coumaroyloxy methylensylloxybutenenitrile]	219, 310	nd	nd	258.0758/258.0771	nd	163.0390, 214.0861	259.0831/259.0844	−5.0	C ₁₄ H ₁₃ NO ₄	UV, QTOF	20	
16	myricetin-3-O-rutinoside	210, 356	627.1587/627.1556	−5	319.0462, 481.0996	625.1320/625.1351	5	nd	626.1393/626.1424	−5.0	C ₂₇ H ₃₀ O ₁₇	UV, QTOF	7,20
17	myricetin-3-O-glucoside	210, 355	481.0977/481.0996	−3.9	319.0461	nd	nd	480.0904/480.0923	−4.0	C ₂₁ H ₂₀ O ₁₃	UV, QTOF	7,20	
18	myricetin-3-O-arabinoside	216, 355	451.0891/451.087	−4.4	319.0458	nd	nd	450.0818/450.0797	4.7	C ₂₀ H ₁₈ O ₁₂	UV, QTOF	20	
19	myricetin-3-O-(6''-malonyl)-galactoside	208, 254	567.0997/567.0981	−2.9	319.046	565.0824/565.0835	2	521.0924	564.0751/564.0762	−2.0		UV, QTOF	20
20	quercetin-3-O-rutinoside	205, 352	611.1622/611.1607	−2.6	465.1037, 303.0505	nd	nd	610.1549/610.1534	2.5	C ₂₇ H ₃₁ O ₁₆	UV, QTOF, STD	20	
21	quercetin-3-O-glucoside	205, 352	465.1032/465.1028	−1	417.1369, 303.0500	nd	nd	464.0959/464.0955	0.9	C ₂₁ H ₂₀ O ₁₂	UV, QTOF, STD	20	
22	kaempferol-3-O-rutinoside	208, 350	595.165/595.1657	1.3	449.1075, 287.0536	593.1438/593.1453	2.5	284.0294	594.1511/594.1526	−2.5	C ₂₇ H ₃₀ O ₁₅	UV, QTOF	20
23	quercetin-3-O-(6''-malonyl)-glucoside	218, 352	551.1026/551.1031	1	303.049	nd	nd	550.0953/550.0958	−0.9	C ₂₈ H ₂₂ O ₁₅	UV, QTOF	20	
24	kaempferol-3-O-galactoside	210, 343	449.1076/449.1078	0.6	287.0549	nd	nd	448.1003/448.1005	−0.4	C ₂₁ H ₂₀ O ₁₁	UV, QTOF	20	
25	myricetin	219, 371	nd	nd	nd	nd	nd	nd	nd		UV	20	
26	quercetin	220, 368	303.0505/303.0499	−1.8	nd	nd	nd	302.0432/302.0426	−2.8	C ₁₅ H ₁₀ O ₇	UV, QTOF	20	
27	epi-galocatechin	210, 287	307.0813/307.0812	−0.1	nd	305.0644/305.0666	7.6	611.1325	306.0717/306.0739	−7.2	C ₁₅ H ₁₄ O ₇	UV, QTOF	1
28	catechin	202, 283	nd	nd	289.0682/289.0718	nd	12.3	465.0979	290.0755/290.0791	−12.4	C ₁₅ H ₁₄ O ₆	UV, QTOF	1
29	epi-catechin	204, 279	291.0871/291.0863	−0.7	nd	nd	nd	290.0798/290.079	2.8	C ₁₅ H ₁₄ O ₆	UV, QTOF	1	
30	delphinidin-3-O-glucoside	277, 523	465.1051/465.1028	nd	nd	nd	nd	464.0978/464.0955	5.0	C ₂₁ H ₂₁ O ₁₂	UV, QTOF	20,36,37	
31	delphinidin-3-O-rutinoside	277, 524	611.1599/611.1607	1.3	nd	nd	nd	610.1526/610.1534	−1.3	C ₂₇ H ₃₁ O ₁₆	UV, QTOF	20	
32	cyanidin-3-O-glucoside	279, 514	449.1083/449.1078	−1.1	nd	nd	nd	448.1010/448.1005	1.1	C ₂₁ H ₂₁ O ₁₁	UV, QTOF	20,36,37	
33	cyanidin-3-O-rutinoside	280, 516	595.166/595.1657	−0.5	nd	nd	nd	594.1587/594.1584	0.5	C ₂₇ H ₃₁ O ₁₅	UV, QTOF	20,36,37	
34	petunidine-3-O-rutinoside	526, 277	625.1758/625.1763	0.9	433.1122	nd	nd	624.1685/624.1690	−0.8	C ₂₈ H ₃₃ O ₁₆	UV, QTOF	35	
35	peonidin-3-O-rutinoside	273, 515	609.1811/609.1814	0.5	nd	nd	nd	608.1738/608.1741	−0.5	C ₂₈ H ₃₃ O ₁₅	UV, QTOF	35	
36	delphinidin 3-O-(6''-coumaroyl)-glucoside	281, 528	611.1368/611.1395	4.5	303.8837	nd	nd	610.1295/610.1322	−4.4	C ₃₀ H ₂₇ O ₁₄	UV, QTOF	7	
	delphinidin	267, 505	nd	nd	nd	nd	nd	nd	nd	nd	C ₁₅ H ₁₁ O ₇	UV	7

Table 2. continued

compound number	tentative identification ^a	MS (m/z) ⁺		MS ²		MS (m/z) ⁻		MS ²		exact mass (molecule)	M (error, ppm)	molecular formula	identification	ref
		[M + H] ⁺ exact mass/calculated mass	[M + H] ⁺ (error ppm)	[M + H] ⁺	[M + H] ⁺ (error ppm)	[M - H] ⁻ exact mass/calculated mass	[M - H] ⁻ (error ppm)	[M - H] ⁺	measurement/calculated					
37	cyranidin 3-O-(6'-coumaroyl)-glucoside	595.143/595.1446	2.8	287.0542	nd	594.1357/594.1373	nd	nd	594.1357/594.1373	-2.7	C ₂₈ H ₃₃ O ₁₅	UV, QTOF	7	
38	cyranidin	nd	nd	nd	nd	nd	nd	nd	nd	nd	C ₁₅ H ₁₁ O ₆	UV	7	
39	cyranidin-3-O-rutinoside pyruvic acid	663.1551/663.1556	0.7	595.1648	nd	662.1478/662.1483	nd	nd	662.1478/662.1483	-0.8	C ₃₀ H ₃₁ O ₁₇	QTOF	37	
40	cyranidin-3-O-rutinoside acetaldehyde	619.1653/619.1657	0.7	595.1584-473.1092	nd	618.158/618.1584	nd	nd	618.158/618.1584	-0.6		QTOF	37	

^aPhenolic compounds were identified using HPLC-DAD-ESI-QTOF by comparing the reference standards and previous literature. nd = not detected.

variables ($n = 57$; first three PCs explain 74% of the total variance) clearly shows that black currant juice is clearly separated from the fermentation samples on the PC2 (panels A and B of Figure 2). Not surprisingly, the ethanol and total sugar contents correlate negatively with one another. However, most of the organic acids and the total organic acid content correlate more positively with fermented beverages than with black currant juice. The nitrile-containing hydroxycinnamic acids (variables 10–15), certain anthocyanins (e.g., rutinosides of delphinidin and cyanidin), and the majority of sugars correlate positively with black currant juice. The aglycones of the nitrile-containing hydroxycinnamic acids, ethanol, and myricetin rutinoside correlated negatively with the black currant juice.

3.5. Comparison of Chemical Composition of Beverages Fermented with Different Yeast Strains. **3.5.1. Organic Acids and Residual Sugars.** Citric acid was the primary organic acid in the black currant beverages. Its concentrations varied between 30.3 g/L (M2Sc1) and 35.2 g/L (M1Sc1). The concentrations of citric acid increased moderately in all of the other fermentations, except Sb and M2Sc1.

The highest concentration of succinic acid was found in the Sb beverage (930 mg/L); the second highest concentration of succinic acid was found in the Td beverage (770 mg/L); and the lowest concentrations were observed in the sequentially fermented beverages. Our results are concurrent with Eglinton et al.,⁴³ who reported that *S. bayanus* produced more succinic acid than *S. cerevisiae* during fermentation. In addition, high production rates of succinic acid by *T. delbrueckii* have been reported before.^{30,33} Canonico et al.⁴⁴ reported increases in the succinic acid concentration when a beverage was fermented with *M. pulcherrima* and *S. cerevisiae*, and Liu et al.⁴⁵ reported similar succinic acid levels in sequential fermentation with *M. fructicola* and *S. cerevisiae*. In our study, the concentrations of succinic acid in the sequentially fermented beverages were significantly lower than they were in the Sc1 and Sc2 beverages. According to Rubico and McDaniel,⁴⁶ the taste properties of succinic acid are sour and bitter at a concentration of 680 mg/L. In the present study, the concentrations of succinic acid in the fermented beverages were above the reported threshold, indicating possible effects on the sensory properties of the beverages.

The highest galacturonic acid concentrations were in the sequential fermentations (280–310 mg/L), and the lowest galacturonic acid concentration was in Sb (160 mg/L). Belda et al.¹⁶ reported that both *M. pulcherrima* and *M. fructicola* have high polygalacturonase activity, and the use of *M. pulcherrima* with *S. cerevisiae* was beneficial for the sensory and technological properties of wine products as a result of the release of important sensory active compounds by pectin degradation.

Clear differences were observed in the contents of residual sugars between the yeasts studied here (Table 1). Sc1 had the lowest amounts of residual glucose (0.08 g/L) and fructose (0.1 g/L), whereas Sb had the highest amount (1.0 and 1.9 g/L, respectively) but the lowest amount of sucrose (0.1 g/L). Sc2 also had a low amount of sucrose left (0.4 g/L), whereas Sc1 had a higher amount (2.8 g/L). Interestingly, sequential fermentations had the highest amount of sucrose left after fermentation (3.5–3.9 g/L), corresponding to approximately half of the initial sucrose concentration and 10 times the level in Sc2. Additionally, no glucose or fructose was detected after the sequential fermentations. Differences in the use of sugar may result from different gene expressions across the yeast strains and species. Generally, yeasts preferably use hexose

Table 3. Contents of Hydroxycinnamic Acids, Flavan-3-ols, Flavonols, Anthocyanins, and Citric Acid Methyl Esters in Black Currant Juice and Fermented Beverages^a

number	compound name	juice	Scl	S _{c2}	S _b	T _d	M1Sc1	M1Sc2	M2Sc1	averaged beverage ^b
Hydroxycinnamic Acids (mg/L)										
1	(<i>E</i>)-caffeoylglucose	19.4 ± 6 ab	19.3 ± 2.4 ab	18.7 ± 1.2 a	19.2 ± 3.0 ab	19.0 ± 2.1 a	21.0 ± 1.7 ab	23.0 ± 1.8 b	20.1 ± 1.6 ab	20.0 ± 2.4
2	<i>p</i> -coumaric acid <i>O</i> -glucoside	1.3 ± 0.5 a	1.5 ± 0.2 ab	1.8 ± 0.2 b	1.9 ± 0.3 b	1.7 ± 0.3 b	1.6 ± 0.1 ab	1.6 ± 0.1 ab	1.7 ± 0.2 b	1.7 ± 0.2
3	1- <i>O</i> -caffeoylglucose	1.8 ± 0.4 abc	1.8 ± 0.2 abc	1.7 ± 0.3 a	1.7 ± 0.2 ab	1.7 ± 0.2 a	2.1 ± 0.2 c	1.8 ± 0.1 abc	2.1 ± 0.2 bc	1.8 ± 0.2
4	coumaroylquinic acid	1.9 ± 0.3 a	1.8 ± 0.2 a	1.9 ± 0.2 a	1.9 ± 0.2 a	1.8 ± 0.1 a	2.0 ± 0.1 ab	2.3 ± 0.1 b	2.0 ± 0.1 a	2.0 ± 0.2
5	4- <i>O</i> -coumaroylglucose	10.9 ± 1.0 a	10.0 ± 1.2 a	10.2 ± 0.5 a	10.2 ± 1.1 a	10.0 ± 1.0 a	11.0 ± 0.6 ab	12.4 ± 0.7 b	10.9 ± 0.5 a	10.7 ± 0.1
6	ferulic acid hexoside	1.9 ± 0.4 ab	1.8 ± 0.2 ab	1.9 ± 0.2 ab	1.9 ± 0.3 ab	1.7 ± 0.2 a	2.0 ± 0.1 b	2.0 ± 0.2 b	1.9 ± 0.2 ab	1.9 ± 0.2
7	(<i>Z</i>)- <i>p</i> -coumaroylglucose	0.7 ± 0.3 a	0.9 ± 0.2 ab	1.1 ± 0.2 bc	1.4 ± 0.3 c	1.1 ± 0.3 bc	0.9 ± 0.09 ab	1.0 ± 0.2 abc	1.1 ± 0.5 bc	1.1 ± 0.3 ^c
8	ferulic acid hexoside	3.0 ± 0.4 a	5.2 ± 0.6 c	5.4 ± 0.3 c	3.7 ± 0.4 b	3.5 ± 0.3 ab	7.2 ± 0.3 e	6.3 ± 0.8 d	7.0 ± 0.4 e	5.5 ± 1.4 ^c
9	<i>p</i> -coumaric acid	1.2 ± 0.1 a	1.5 ± 0.3 a	4.6 ± 0.4 e	2.1 ± 0.2 b	2.6 ± 0.3 c	4.5 ± 0.2 e	4.2 ± 0.5 e	3.1 ± 0.4 d	3.3 ± 1.2 ^c
10	(<i>E</i>)-caffeoyloxymethylene eglucosyloxybutanenitrile	3.0 ± 0.2	nd	nd	nd	nd	nd	nd	nd	nd ^c
11	(<i>E</i>)- <i>p</i> -coumaroyloxymethylene glucosyloxybutanenitrile	7.3 ± 0.5 d	0.5 ± 0.1 a	0.5 ± 0.2 a	2.0 ± 0.6 c	0.9 ± 0.3 a	1.6 ± 0.1 b	0.5 ± 0.07 a	0.5 ± 0.1 a	0.9 ± 0.6 ^c
12	(<i>Z</i>)- <i>p</i> -coumaroyloxymethylene glucosyloxybutanenitrile	1.4 ± 0.2 d	0.5 ± 0.01 a	0.5 ± 0.06 ab	0.9 ± 0.2 c	0.9 ± 0.09 b	0.6 ± 0.02 ab	0.05 ± 0.03 a	0.6 ± 0.1 ab	0.6 ± 0.1 ^c
13	(<i>E</i>)-feruloyloxymethylene glucosyloxybutanenitrile	5.1 ± 0.4 c	0.3 ± 0.01 a	0.3 ± 0.06 a	0.8 ± 0.2 b	0.4 ± 0.1 a	0.7 ± 0.05 b	0.3 ± 0.05 a	0.3 ± 0.05 a	0.4 ± 0.2 ^c
14	(<i>E</i>)- <i>p</i> -coumaroyloxymethylene oxybutanenitrile	nd	3.5 ± 0.4 b	3.4 ± 0.4 b	2.9 ± 0.3 ab	2.7 ± 0.5 a	5.3 ± 0.3 d	4.8 ± 0.7 cd	4.4 ± 0.3 c	3.8 ± 1.0 ^c
15	(<i>Z</i>)- <i>p</i> -coumaroyloxymethylene oxybutanenitrile	nd	0.44 ± 0.08 a	0.5 ± 0.07 a	0.5 ± 0.07 a	0.4 ± 0.09 a	0.7 ± 0.04 b	0.7 ± 0.04 b	0.6 ± 0.1 b	0.5 ± 0.1 ^c
	total	59 ± 9 cd	54 ± 3 a	57 ± 3 abc	55 ± 7 ab	53 ± 4 a	68 ± 4 d	67 ± 5 d	63 ± 3 bcd	59.5 ± 7 ^c
Flavan-3-ols (mg/L)										
16	epi-gallocatechin	11 ± 3 ab	10 ± 1 a	11 ± 1 ab	12 ± 2 b	11 ± 1 ab	13 ± 1 b	13 ± 1 b	13 ± 1 b	12 ± 2
17	catechin	35 ± 10	36 ± 4	38 ± 2	39 ± 5	38 ± 4	41 ± 3	41 ± 3	40 ± 4	39 ± 4
18	PB-2	4.8 ± 0.2	4 ± 0.8	4.5 ± 1.3	4.9 ± 0.8	3.9 ± 0.1	4.1 ± 0.1	4.0 ± 0.3	4.3 ± 0.6	4.0 ± 1
	total	50 ± 14	50 ± 6	53 ± 4	57 ± 7	53 ± 6	58 ± 3	57 ± 4	57 ± 5	55 ± 6
Flavonols (mg/L)										
19	myricetin rutinoside	4.8 ± 2.1 a	6.8 ± 0.5 b	7.5 ± 0.3 b	7.4 ± 1.1 b	7.2 ± 1.0 b	7.2 ± 0.5 b	7.2 ± 0.7 b	7.2 ± 0.9 b	7 ± 1 ^c
20	myricetin glucoside	24.4 ± 4.2	24.7 ± 0.6 a	27.3 ± 1.7 a	26.9 ± 2.8 a	24.6 ± 2.3 a	33.0 ± 1.5 b	28.2 ± 2.5 a	34.5 ± 3.4 b	28 ± 4 ^c
21	myricetin arabinoside	0.7 ± 0.04 c	0.5 ± 0.03 ab	0.6 ± 0.1 ab	0.6 ± 0.1 ab	0.5 ± 0.1 ab	0.5 ± 0.03 bc	0.5 ± 0.04 ab	0.6 ± 0.1 ab	1 ± 0.1 ^c
22	myricetin malonylgalactoside	8.2 ± 0.7 a	7.6 ± 0.2 a	8.1 ± 0.8 a	8.0 ± 0.8 a	7.5 ± 0.9 a	10.7 ± 0.6 bc	9.6 ± 0.9 b	11.1 ± 1.1 c	9 ± 2
23	quercetin rutinoside	10.0 ± 3.0 a	10.3 ± 0.5 ab	11.3 ± 0.5 ab	11.7 ± 1.4 ab	10.9 ± 1.3 ab	11.7 ± 0.7 ab	11.8 ± 1.0 ab	12.0 ± 1.0 b	11 ± 1
24	quercetin glucoside	18.9 ± 0.5 a	17.3 ± 0.4 a	18.7 ± 1.3 a	18.4 ± 1.9 a	16.9 ± 1.0 a	23.0 ± 1.0 b	22.2 ± 1.6 b	23.9 ± 1.8 b	20 ± 3 ^c
25	kaempferol rutinoside	6.4 ± 0.3 ab	5.4 ± 0.2 a	6.1 ± 0.4 a	5.9 ± 0.6 a	5.4 ± 0.6 a	7.5 ± 0.5 c	7.2 ± 0.6 bc	7.7 ± 0.8 c	6 ± 1
26	quercetin malonylgalactoside	0.7 ± 0.03 ab	0.9 ± 0.1 ab	0.9 ± 0.3 ab	0.5 ± 0.1 a	1.0 ± 0.1 ab	0.9 ± 0.2 ab	0.7 ± 0.1 ab	0.9 ± 0.1 ab	1 ± 0.4
27	kaempferol galactoside	4.5 ± 0.5 b	3.7 ± 0.1 a	3.8 ± 0.3 a	3.9 ± 0.4 a	3.6 ± 0.2 a	5.5 ± 0.3 c	4.8 ± 0.3 b	5.6 ± 0.4 c	4 ± 1
28	myricetin	1.5 ± 0.1 b	1.3 ± 0.1 ab	1.3 ± 0.2 ab	1.3 ± 0.2 ab	1.2 ± 0.1 a	2.0 ± 0.1 c	1.3 ± 0.2 ab	2.3 ± 0.3 d	2 ± 0.4
29	quercetin	0.8 ± 0.1 b	0.5 ± 0.03 a	0.6 ± 0.1 a	0.5 ± 0.1 a	0.4 ± 0.1 a	1.2 ± 0.07 c	0.8 ± 0.1 b	1.2 ± 0.1 c	1 ± 0.3
	total	81 ± 10 a	79 ± 2 a	86 ± 5 ab	85 ± 9 ab	79 ± 7 a	103 ± 5 cd	94 ± 8 bc	107 ± 10 d	90 ± 12
Anthocyanins (mg/L)										
30	delphinidin glucoside	138 ± 4 d	87 ± 15 b	93 ± 13 b	61 ± 19 a	94 ± 8 b	125 ± 28 cd	111 ± 17 bc	132 ± 16 cd	95 ± 34
31	delphinidin rutinoside	455 ± 12 d	323 ± 26 b	345 ± 47 b	221 ± 31 a	348 ± 57 bc	422 ± 53 cd	396 ± 62 bcd	433 ± 60 cd	335 ± 110

Table 3. continued

number	compound name	juice	Sc1	Sc2	Sb	Td	M1Sc1	M1Sc2	M2Sc1	averaged beverage ^b
32	cyanidin glucoside	77 ± 2 e	40 ± 3 ab	43 ± 6 ab	32 ± 4 a	48 ± 9 b	61 ± 8 cd	51 ± 8 bc	64 ± 10 d	46 ± 16
33	cyanidin rutinoside	463 ± 12 d	322 ± 25 b	345 ± 49 b	220 ± 30 a	346 ± 58 bc	430 ± 54 cd	393 ± 62 bcd	445 ± 62 d	336 ± 113
34	petunidin rutinoside	12 ± 0.3 d	9 ± 0.7 abc	9 ± 1 ab	6 ± 0.8 a	9 ± 1 bc	11 ± 1 cd	10 ± 2 bcd	11 ± 1 cd	9 ± 3
35	peonidin rutinoside	8.9 ± 0.2 c	5.6 ± 1.9 ab	12 ± 2 ab	4.4 ± 0.6 a	6.7 ± 1.0 abc	8.2 ± 1.0 bc	6.5 ± 3 ab	8.3 ± 1.1 bc	6 ± 3
36	delphinidin 3-O-(6-p-coumaroyl) glucoside	3.7 ± 0.1 e	2.4 ± 0.2 b	3 ± 0.4 b	1.6 ± 0.2 a	2.5 ± 0.5 bc	3.0 ± 0.4 cd	2.8 ± 0.4 bcd	3.2 ± 0.5 de	2 ± 1
37	delphinidin (aglycone)	nd	0.9 ± 0.1 b	nd	0.2 ± 0.05 a	0.2 ± 0.1 b	0.1 ± 0.06 bc	nd	0.3 ± 0.06 c	0.3 ± 0.05 ^c
38	cyanidin 3-O-(6-p-coumaroyl) glucoside	1.6 ± 0.4 d	1.0 ± 0.1 b	1.0 ± 0.2 b	0.1 ± 0.2 b	0.7 ± 0.9 a	1.2 ± 0.2 bc	1.2 ± 0.2 c	1.2 ± 0.14 bc	1 ± 0.3
39	cyanidin (aglycone)	0.07 ± 0.01 a	0.9 ± 0.1 d	0.1 ± 0.02 a	0.3 ± 0.1 bc	0.3 ± 0.1 bc	0.2 ± 0.07 ab	nd	0.4 ± 0.05 c	0.3 ± 0.01 ^c
	total	1159 ± 30 c	905 ± 207 abc	873 ± 81 ab	637 ± 144 a	821 ± 159 ab	1153 ± 113 c	765 ± 272 ab	989 ± 145 bc	830 ± 280
40	citric acid methyl esters (mg/L)	67 ± 2. ab	62 ± 8 a	67 ± 4 ab	66 ± 5 ab	63 ± 2 ab	91 ± 2 c	71 ± 4 b	89 ± 9 c	73 ± 12 ^c

^aBC, black currant; Sc1, *S. cerevisiae* 1; Sc2, *S. cerevisiae* 2; Sb, *S. bayanus*; Td, *T. delbrueckii*; M1Sc1 *M. pulcherrima* and *S. cerevisiae* 1; M1Sc2 *M. pulcherrima* and *S. cerevisiae* 2; and M2Sc1 *M. fructicola* and *S. cerevisiae* 1. The results are expressed as the average of three biological replicates ± standard deviation. The contents of phenolic acids are expressed as equivalents of p-coumaric acid (310 nm) or chlorogenic acid (320 nm); the contents of flavan-3-ols are expressed as equivalents of (+)-catechin; the contents of flavonols are expressed as equivalents of quercetin-3-O-glucoside; and the contents of anthocyanins are expressed as equivalents of cyanidin-3-O-glucoside. The same letters in each row indicate that there is no significant ($p > 0.05$) difference between samples. ^bAveraged beverage was calculated from all of the biological replicates of fermentations. ^cStatistically significant difference between black currant juice and averaged beverage ($p < 0.05$).

monosaccharides, such as glucose and fructose, because these sugars enter the glycolytic pathway directly. To degrade sucrose, yeasts have to first produce the invertase enzyme. High concentrations of glucose suppress the expression of the genes encoding the invertase enzyme. Therefore, when glucose is present at high levels, the production of invertase is inhibited by yeast.⁴⁷

3.5.2. Phenolic Acids, Flavonols, and Anthocyanins. The phenolic acid contents in the fermented samples varied between 53 and 67 mg/L (Table 3). In general, the total phenolic acid contents were significantly lower in all of the single yeast fermentations compared to the sequential fermentations.

Six phenolic acids containing nitrile groups (compounds 10–15 in Table 2) were detected in the black currant juice and beverages. The contents of nitrile compounds decreased 1.5–19-fold (Table 3) during the fermentations, and one of the nitrile compounds was detected only in trace amounts. Two new nitrile compounds were detected in the fermented black currant beverages, which were tentatively identified as aglycones of the compounds (*E*)-coumaroyloxymethyleneglycosyloxybutenenitrile and (*Z*)-coumaroyloxymethyleneglycosyloxybutenenitrile (section 3.3). The concentrations of these compounds differed significantly between fermentations; the sequentially fermented beverages had significantly higher contents of both compounds than single yeast-fermented beverages. This result is consistent with the reported high β -glucosidase activity of *Metschnikowia* yeasts.⁴⁸ In addition, the concentrations of the initial compounds (11 and 12) decreased 1.5–15-fold. The highest decrease was observed with Sc1 by 93.5 and 65%, respectively.

The main flavonols in the black currant juice and beverages were glycosides of myricetin and quercetin. Interestingly, sequential fermentations did have different degradation effects on the flavonols: M1Sc1 and M2Sc1 significantly increased the flavonol aglycon contents, but M1Sc2 did not increase them at all. The total flavonol glycoside contents varied between 79 mg/L (Sc1) and 107 mg/L (M2Sc1; Table 3) in the fermented beverages. The highest content was found in M1Sc1 and M2Sc1, which differed significantly with the one Sc1 beverage, but there were no significant differences between the M1Sc2 and Sc2 beverages. The yeast fermentations did not have a significant effect on the contents of flavan-3-ols. A moderate increase in the catechin content was observed after the sequential fermentations (Table 3).

The color properties, intensity, and tonality of the fermented beverages were analyzed with a spectrophotometer (Table 1). The highest color intensity was recorded from black currant juice. Of the beverages, the Sc2-fermented beverage had the highest color intensity and the Sb beverage had the lowest color intensity. The colors of the beverages produced by sequential fermentations were recorded as more intense than the Sc1, Sb, and Td beverages. A decreased blue color proportion and increased proportion of yellow and red colors were detected in beverages with lower color intensity and lower total anthocyanin contents. After the fermentations, the total anthocyanin contents varied between 637 mg/L (Sb) and 1153 mg/L (M1Sc1; Table 3). Clear differences in the contents of individual monomeric anthocyanins and the total anthocyanins were observed between the sequential and non-sequential fermentations. The total anthocyanin contents of the sequential fermentations did not differ statistically from the non-fermented black currant juice or from each other, whereas all of the fermentations with single yeast strains did. Sb had the lowest total anthocyanin content. The difference between fermentations was in the extent of the decrease in all of the monomeric anthocyanins. Similar

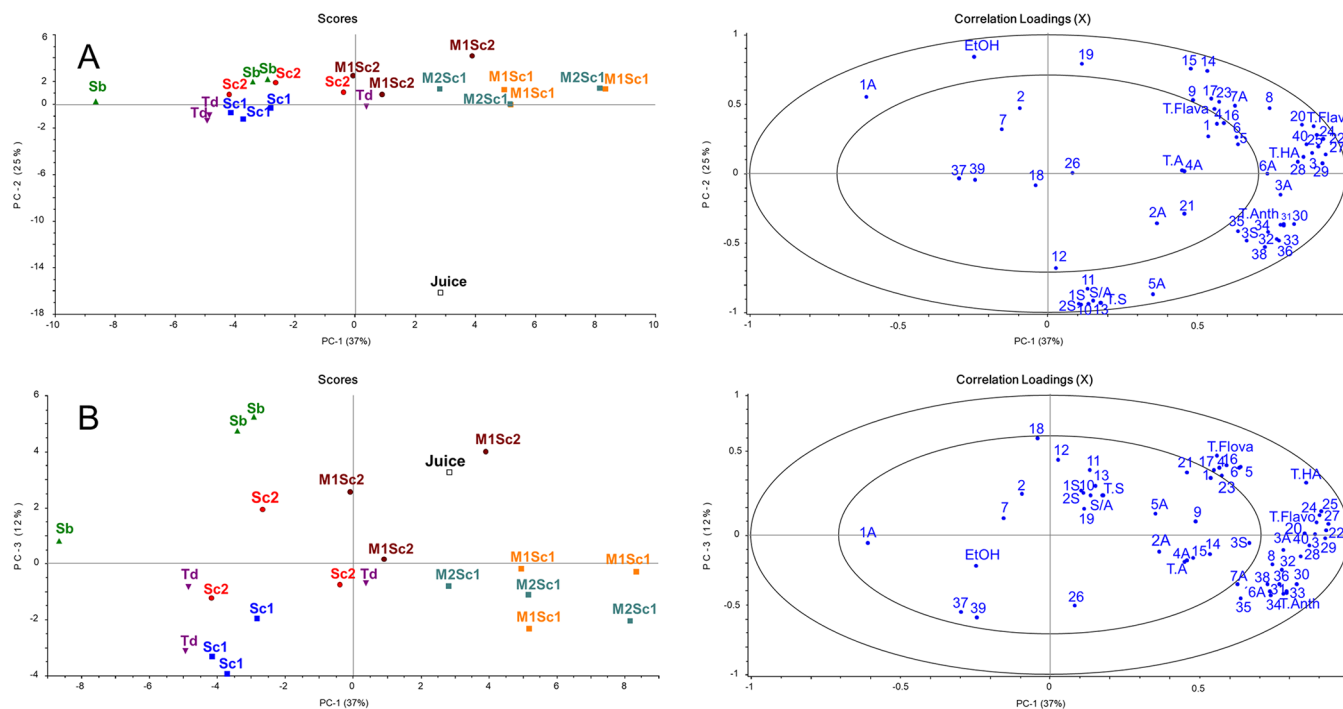


Figure 2. PCA based on 57 chemical variables in black currant juice and fermented black currant beverages using the following inoculation schemes: (black \square) black currant juice, (blue \blacksquare) *S. cerevisiae* 1, (red \bullet) *S. cerevisiae* 2, (green \blacktriangle) *S. bayanus*, (purple \blacktriangledown) *T. delbrueckii*, (orange \blacksquare) *M. pulcherrima* + *S. cerevisiae* 1, (cyan \blacksquare) *M. fructicola* + *S. cerevisiae* 1, and (maroon \bullet) *M. pulcherrima* + *S. cerevisiae* 2 for (A) PC1 versus PC2, and (B) PC1 versus PC3. Numbers with A (acid) or S (sugar) refer to Table 1, and the numbers of variables refer to Table 3. T.HA, total hydroxycinnamic acids; T.Flava, total flavan-3-ols; T.Flavo, total flavonols; T.Anth, total anthocyanins; T.A, total organic acids; T.S, total sugars; and S/A, sugar/acid ratio.

to our findings, Belda et al. and Escribano-Viana et al.^{10,16} have reported higher anthocyanin contents after sequential fermentation with *M. pulcherrima* and *S. cerevisiae* compared to *S. cerevisiae* fermentation. The observed differences in color properties and the loss of monomeric anthocyanin during the fermentations can be attributed to variations in either β -glucosidase activity or anthocyanin absorption by the yeast cell wall among different yeast strains. β -Glucosidase releases the aglycon forms of anthocyanins through the cleavage of the glycosidic bond. Aglycons are not stable and spontaneously convert to colorless and brown compounds. Both β -glucosidase activity and cell wall absorption are yeast species and strain-specific characteristics.^{14,49–51} Belda et al.¹⁶ reported β -glucosidase activity in 88.5% of the studied *M. pulcherrima* strains and 88.9% of *M. fructicola* strains.

To visualize the relationship between single yeast fermentations and sequential fermentations and between *Saccharomyces* and non-*Saccharomyces* beverages, a second PCA was constructed using all 21 fermented beverage samples and 57 chemical variables. The first three PCs are shown in panels A and B of Figure 3 and explain 70% of the data variance. PC1 clearly separates the single yeast fermentations from the sequential fermentations. Sequential fermentations are located on the left side of the scores plot, and the single yeast-fermented beverages are on the right. Between sequential fermentations, there are still clear differences, with M1Sc1 and M2Sc1 located closer to each other than to M1Sc2. M1Sc2 is also separated from M1Sc1 and M2Sc1 on PC2. The correlation loadings plot shows almost all of the chemical variables, especially flavonols and anthocyanins, located on the left side with the Sc1 sequential fermentations. Galacturonic acid (7A) correlates positively with M1Sc2 beverages and clustering most of the anthocyanins and total anthocyanins.

This classification can indicate higher pectinase enzyme activity and, thus, positive effects on the contents of phenolic compounds after fermentation. Sc1, Sc2, Td, and Sb beverages are primarily on the right side of the plot. However, the Sb beverages are separated from other single yeast-fermented beverages on PC2. In addition, the majority of chemical variables are correlated positively with Sb, such as *Z-p*-coumaroyl *O*-glucoside (6), *p*-coumaric acid *O*-glucoside (3), succinic acid (1A), fructose (1S), and glucose (2S). The scores plot also shows that the Sc1, Sc2, and Td beverages are not separated from each other by the differences in the studied chemical variables, and thus, their sensory properties may be more similar to each other than to Sb and sequentially fermented beverages. The only chemical variables positively correlating with Sc1, Sc2, and Td are the delphinidin and cyanidin aglycones and ethanol. The third component separates the M1Sc2 samples from other sequential fermentations and brings the Sb beverages closer to other single yeast-fermented beverages. Additionally, the Sc1 and Sc2 beverages are separated from Td and Sb, indicating that they are more similar to each other than to the other two single yeast fermentations. However, the chemical variables are positively correlated more with Sb and Td. Most interestingly, M1Sc2 clearly correlates negatively with the anthocyanins, initial nitrile compounds (10–13), glucose, fructose, and succinic acid on PC3. The difference between biological replicates of fermentations may be caused by the high pectin content of the black currant juice.

In conclusion, although black currant berries and juices are widely studied,^{3,6,7,20} studies on the composition of black currant fermented beverages are scarce and focused only on fermentations with *S. cerevisiae*.^{21,22,24,25,27} To our knowledge, this is the first study on the effects of non-*Saccharomyces* yeast fermentations on the chemical composition of the fermented black

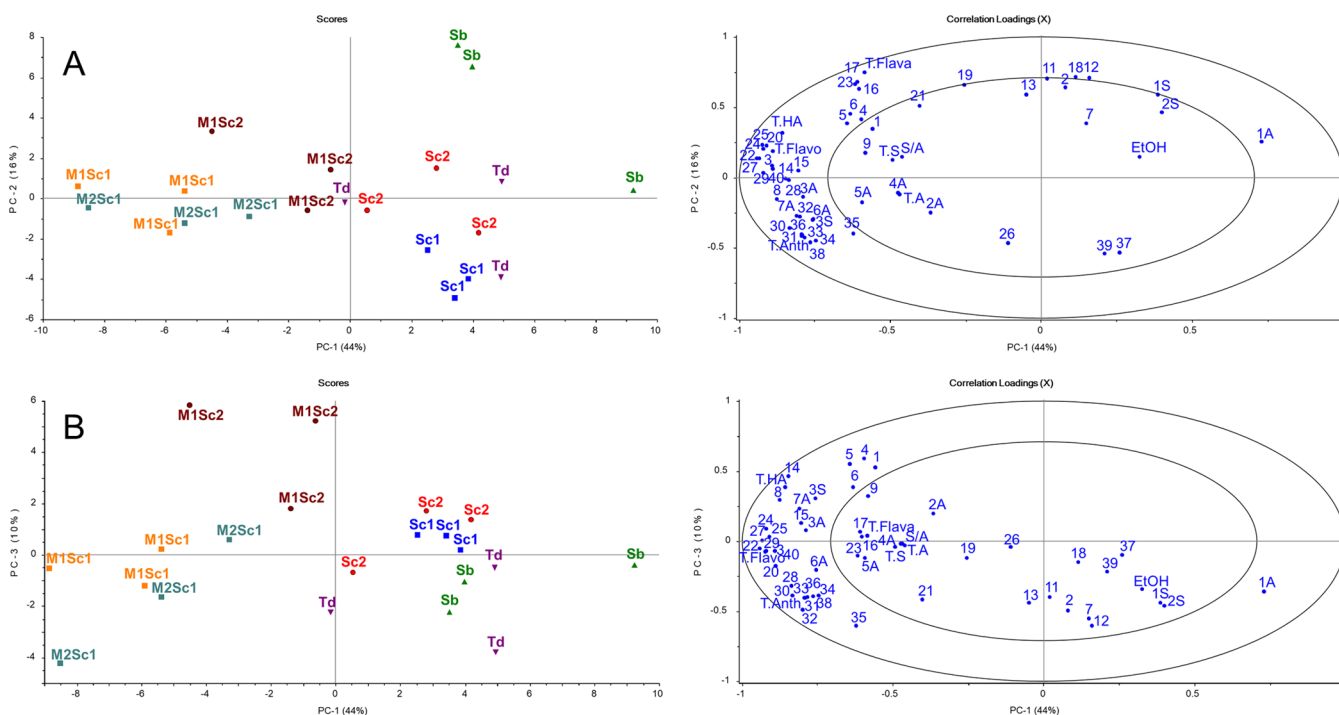


Figure 3. PCA based on 57 chemical variables of fermented black currant beverages using the following inoculation schemes: (blue ■) *S. cerevisiae* 1, (red ●) *S. cerevisiae* 2, (green ▲) *S. bayanus*, (purple ▼) *T. delbrueckii*, (orange ■) *M. pulcherrima* + *S. cerevisiae* 1, (cyan ■) *M. fructicola* + *S. cerevisiae* 1, and (maroon ●) *M. pulcherrima* + *S. cerevisiae* 2 for (A) PC1 versus PC2 and (B) PC1 versus PC3. Numbers with A (acid) or S (sugar) refer to Table 1, and the numbers of variables refer to Table 3. T.HA, total hydroxycinnamic acids; T.Flava, total flavan-3-ols; T.Flavo, total flavonols; T.Anth, total anthocyanins; T.A, total organic acids; T.S, total sugars; and S/A, sugar/acid ratio.

currant beverages. All of the fermentations decreased the content of phenolic acids containing nitrile moieties. Sequential fermentations increased the contents of hydroxycinnamic acids, flavan-3-ols, and flavonols but decreased the ethanol content compared to single yeast fermentations. Beverages fermented with *S. cerevisiae* or *T. delbrueckii* alone were similar to each other, whereas *S. bayanus* was significantly different from the other single yeast-fermented beverages. The *S. bayanus* beverage had the lowest amounts of anthocyanins, and its color intensity was significantly lower than that of all of the other fermented beverages. When three sequential fermentations were compared to each other, it was clear that the *S. cerevisiae* strain also effects the concentrations of phenolic compounds; fermentations with either *Metschnikowia* yeast or *S. cerevisiae* strain 1 resulted primarily in more of phenolic compounds than fermentation with *M. pulcherrima* and *S. cerevisiae* strain 2. Sequential fermentation with *Metschnikowia* and *S. cerevisiae* yeasts could be considered to be the most suitable for black currant juice, if the aim is to maximize the preservation of the phenolic compounds and to gain the highest sugar/acid ratio. In this sense, single yeast fermentations would be more suitable for higher ethanol production. In addition, *S. bayanus* was the least suitable yeast to ferment black currant juice as a result of its high succinic acid production, low phenolic compound concentrations, and clear decrease in color properties.

This study demonstrated the potential use of *Saccharomyces* and non-*Saccharomyces* yeasts in modifying the chemical composition of black currant beverages. However, there is still a gap in knowledge about how fermentations with different yeasts affect the sensory properties of fermented black currant beverages. Moreover, the fermented black currant beverages could be intensely sour as a result of the sugar utilization by the yeasts and increased

acid content. Further studies are necessary to study how wine yeast fermentations affect the aroma profile of black currant juice.

AUTHOR INFORMATION

Corresponding Author

Niina Kelanne – Food Chemistry and Food Development, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland; orcid.org/0000-0001-7962-0584; Phone: +358-443390661; Email: niina.m.kelanne@utu.fi

Authors

Baoru Yang – Food Chemistry and Food Development, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland; orcid.org/0000-0001-5561-514X

Laura Liljenbäck – Food Chemistry and Food Development, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland

Oskar Laaksonen – Food Chemistry and Food Development, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland; orcid.org/0000-0003-2467-8564

Complete contact information is available at:

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