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Chemical composition, sensory profile and antioxidant capacity of low-alcohol strawberry beverages fermented with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*

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ABSTRACT

There is an increasing demand by consumers for low-alcohol beverages with enhanced flavors and potential health benefits. This study evaluated the effects of cultivars and fermentation with *Saccharomyces cerevisiae* or *Torulaspora delbrueckii* on the chemical composition, antioxidant capacity and sensory properties of low-alcohol strawberry beverages. Compared to juice, fermentation increased the contents of ethyl esters but reduced the contents of anthocyanins (~60%) and organic acids (~50%). The cultivar was the most important factor affecting the physicochemical characteristics and antioxidant capacity of fermented beverages. Among the cultivars, the beverages from the 'Honeoye' presented the highest sourness, opacity, redness and color density, total anthocyanins and phenolics, and antioxidant capacity but the lowest pungent aroma and ethanol content. Without added sugar, there was no significant difference in ethanol content between fermented beverages produced by *S. cerevisiae and T. delbrueckii*. Compared to *S. cerevisiae,* fermentation involving *T. delbrueckii* resulted in higher contents of anthocyanins and enhanced the color and flavor of the beverages. The results indicated that strawberry cultivars should be considered to produce fermented beverages with consistent physicochemical and sensory properties, and *T. delbrueckii* can be an alternative yeast for producing low-alcohol beverages from strawberries.

1. Introduction

Garden strawberry (*Fragaria* × *ananassa*) is the most consumed berry worldwide (https://www.eurofresh-distribution.com/news/record-sales-european-berry-market) due to its delicate taste and aroma, as well as its high content of bioactive compounds with potential beneficial effects on human health. Strawberry is rich in micronutrients and phytochemicals, such as vitamin C and phenolic compounds, which exhibit numerous biological activities, such as antioxidant, anti-inflammatory and prebiotic activities (Ezzat-Zadeh et al., 2021; Giampieri, Alvarez-Suarez, & Battino, 2014). Short-term consumption of strawberries can be associated with improvements in cardiovascular health in adolescent males (Holt et al., 2020).

In Europe, strawberry is the largest cultivated berry crop, and its production has increased to 1.68 million tons (FAOSTAT, 2018). Strawberry is a highly perishable fruit and deterioration leads to substantial economic losses. Therefore, processing strawberries into new, longer-lasting products not only makes full use of valuable resources but also creates added value and greatly improves the economic benefits of the industry and producers. Bioprocessing fermentation presents a sustainable approach for preparing food products with special nutritional and organoleptic qualities (Voidarou et al., 2021). With increasing awareness of health and the requirement for sustainable diets, consumers demand low-alcohol beverages due to the negative effects of high consumption of alcohol. Commercial interest has also been stimulated by the potential low taxes/tariffs on the reduced alcohol content in these classes of fruit wines. However, consumers who are accustomed to traditional wine have generally shown low acceptance of low-alcohol products (Bucher, Deroover, & Stockley, 2018; Pickering, 2000). Hence, it is necessary to investigate the composition of flavor-active compounds

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and improve the sensory qualities of low-alcohol beverages.

Yeasts play an important role in affecting the chemical composition and sensory characteristics of alcoholic beverage products. Yeast strains significantly influence both the concentration and composition of anthocyanins and tannins in wine; moreover, yeast metabolites strongly affect the flavor complexity and mouth feel of wines (Carew, Smith, Close, Curtin, & Dambergs, 2013; Shi, Wang, Chen, & Zhang, 2019). The most of *Saccharomyces cerevisiae* strains have been developed for grape wines, but less is used for making fruit or berry wines. Due to the high risk of spoilage fermentation, non-*Saccharomyces* yeasts were originally considered unsuitable for winemaking. However, recently, multiple advantages have been claimed for non-*Saccharomyces* yeasts compared to conventional *S. cerevisiae* strains, e.g., attention has been focused on *Torulaspora delbrueckii*, as this yeast can enhance flavor and reduce the production of undesirable compounds in fermented beverage products (Canonico, Agarbati, Comitini, & Ciani, 2016; Liu, Laaksonen, Kortesniemi, Kalpio, & Yang, 2018). Although some non-*Saccharomyces* strains are slow growing, possess low to moderate alcoholic fermentation abilities, and able to produce off flavors, the strains can contribute to the sensory characteristics of beverages when appropriate species and fermentation methods are employed.

Although the alcoholic and gluconic (*Gluconobacter japonicus*) fermentations of strawberry fruit have been previously studied (Álvarez-Fernández, Cerezo, Can~ete-Rodríguez, Troncoso, & García--Parrilla, 2015; Álvarez-Fernández, Hornedo-Ortega, Cerezo, Troncoso, & García-Parrilla, 2014), the use of non-*Saccharomyces* yeasts has been less frequently investigated in the strawberry fermentation. Moreover, the effect of fermentation on the chemical composition and sensory properties of strawberry fermented beverages using *T. delbrueckii* strains is still unknown. The research aimed to investigate the chemical composition, sensory characteristics and antioxidant activity of low-alcohol strawberry beverages obtained by fermentation involving *S. cerevisiae* and *T. delbrueckii* without the addition of sugar. The relationship between chemical composition and antioxidant activity was investigated in fermented beverages.

2. Materials and methods

2.1. Strawberry cultivars and yeast strains

Garden strawberry (*Fragaria* × *ananassa*) fruits of three cultivars, were collected in June ('Honeoye') and July ('Polka' and 'Lumotar') 2019 in the open-air test field of Natural Resources Institute Finland (Luke) in Kaarina, southern Finland (60°23′N, 22°33′E). The berries were harvested when optimally ripe for market and transportation, defined by experienced garden personnel. All of the berries, with calyces retained, were stored at −20 °C immediately after collection until processing. Two different yeast strains, *Saccharomyces cerevisiae* Lalvin V1116 (SC, Lallemand Inc., Montreal, Canada) and *Torulaspora delbrueckii* Biodiva (TD, Level™, Edwardstown, Australia), were assessed in this study.

2.2. Preparation of strawberry juices and fermented beverages

Frozen strawberry fruit was thawed in a microwave oven (MW201, Whirlpool, China) at 160 W, and the juice was prepared from ~3 kg of strawberries of each cultivar using a centrifugal juice press (Vita Pro-Active JE810; Kenwood). The cloudy juices were treated with Pectinex® Ultra SP-L (Novozymes, Denmark) (0.25%, v/v) and incubated for approximately 20 min at room temperature. The enzyme-treated juice (juice samples) was pasteurized in a hot water bath (97 °C) for 30 s, measured with a thermometer, and then immediately cooled to room temperature with an ice bath. For each fermentation, 250 mL of pasteurized strawberry juice was immediately transferred to a 500 mL sterile Duran bottle.

According to the instructions for the two yeasts, each accurately weighed yeast strain was rehydrated at 37 °C for 8–10 min using small amounts of pasteurized juice. All fermentations were performed in duplicate and carried out in the dark at room temperature (21 ± 1 °C). Fermentation kinetics were estimated by monitoring the amount of CO_2 released from the weight loss of fermentation bottles every day. Fermentation was considered completed when the °Brix values reached constant levels and no CO_2 production from yeast growth for three consecutive days. After fermentation, the samples were centrifuged at 2880×*g* for 10 min to remove yeast cells and precipitates. A representative sample was taken from each juice and fermented beverage, and stored in 50 mL plastic tubes at −20 °C until analysis. All juices and fermented beverages were prepared in food-grade conditions.

2.3. pH, °Brix and color parameters

The pH values and °Brix were measured by a pH meter (Wissenschaftlich Technische Werkstätten, Weilheim, Germany) and a portable °Brix meter (Atago Co. Ltd., Tokyo, Japan), respectively. The absorbance values of all samples (juices and fermented beverages) at 420, 520 and 620 nm were determined by a plate reader (Hidex, Finland) with a 96-well microplate. The hue was defined as the ratio (A_{420nm}/A_{520nm}), and color density was recorded as the sum ($A_{420nm} + A_{520nm} + A_{620nm}$). Percentages of yellow (%Yellow), red (%Red) and blue (%Blue) elements were expressed as the ratios of absorbance at 420, 520 and 620 nm to color density, respectively (Liu et al., 2018).

2.4. Quantitative analysis of sugars, organic acids and ethanol

Individual sugars and organic acids were analyzed in duplicate as trimethylsilyl (TMS) derivatives by gas chromatography (GC-2010plus, Shimadzu, Japan) equipped with a flame ionization detector (FID) using the method described previously (Ma et al., 2017). The analytes were identified by comparing the retention times of the reference compounds for sugars and organic acids and quantified using an internal standard method.

Ethanol of fermented beverages was determined in duplicate by GC-FID (GC-2010plus, Shimadzu, Japan) as described previously (Liu et al., 2018). Ethanol was quantified using a calibration curve constructed by analysis of ethanol at different concentrations (0–9.95%, $R^2 = 0.996$).

2.5. Analysis of anthocyanins

Anthocyanins were analyzed by HPLC-DAD as described previously (Kelanne et al., 2019). Prior to analysis, 1 mL of the juice/beverage samples was diluted with 1 mL of acidified methanol and filtered through a 0.22 μm PTFE filter. Pelargonidin-3-*O*-glucoside (≥95%, Extrasynthese, Genay, France) was used to construct a calibration curve ($R^2 = 0.999$) to quantify the anthocyanins. Identification of the anthocyanins was carried out using Bruker Elute UHPLC systems as described previously (Liu, Marsol-Vall, Laaksonen, Kortesniemi, & Yang, 2020). The column, eluents and gradient programs were the same as in HPLC-DAD analysis. The flow was split to 0.5 mL/min to the TOF spectrometer. Compass DataAnalysis software 4.4 (Bruker Daltonik) was applied to analyze the mass spectra data. The identification of pelargonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside was based on their corresponding authentic standards by comparing retention time, UV–vis and mass spectrometry information. The other anthocyanin compounds were tentatively identified by matching UV spectra and fragmentation patterns of compounds to data reported in the literature.

2.6. Analysis of free ellagic acid and ellagitannins

The samples prepared for anthocyanin determination were also used for the analysis of free ellagic acid. Ellagitannins (ETs) were determined as ellagic acid (EA) equivalents after acidic hydrolysis using a previous method with a slight modification (Koponen, Happonen, Mattila, &

Törrönen, 2007). Briefly, a 2 mL sample was mixed with 8 mL of methanol, 2 mL of water and 2 mL of concentrated hydrochloric acid. The prepared solution was refluxed at 85 °C for 20 h. After hydrolysis, the solution was cooled to room temperature, rediluted to 20 mL with methanol, and then filtered through a 0.45 μm PTFE filter for HPLC analysis.

The HPLC equipment system and column were the same as previously described for anthocyanin analysis. The mobile phase and gradient program were the same as described by Koponen et al. (2007). Ellagic acid was identified by an authentic standard, and its derivative was identified by UV spectra, retention time and literature data. They were quantified using a calibration curve ($R^2 = 0.999$) constructed by analysis of EA (≥95%, Sigma-Aldrich) at different concentrations.

2.7. Analysis of volatile compounds by HS-SPME-GC-MS

The determination of volatile compounds in strawberry samples was carried out using HS-SPME-GC-MS according to a previously published method (He et al., 2020). Briefly, 2 g of sample and 10 μL of internal standard (4-methyl-2-pentanol at 80.2 μg/mL in methanol) were placed in a 20 mL glass vial. Identification of volatiles was carried out by comparing experimental mass spectrometry data with the NIST10 database and NIST Webbook. Moreover, linear retention indices (RIs) were calculated according to the Van den Dool & Kratz equation using an *n*-alkane mixture (C7–C30). TraceFinder 4.1. (Thermo Scientific) was used to perform peak detection and integration of peak area, which was carried out using the total ion chromatogram (TIC). All individual compounds identified were semiquantified (relative abundance) by comparing their peak areas to that of the internal standard (He et al., 2020).

2.8. Determination of total phenolic content and in vitro antioxidant capacity

The total phenolic content (TPC) of the strawberry juices and fermented beverages (diluted 1:50 with water) were determined by Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) according to the method reported in the literature (Everette et al., 2010). The *in vitro* antioxidant activity was determined by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay and ferric-reducing antioxidant power (FRAP) assay. The DPPH assay was performed using a previously reported method with a slight modification (Álvarez-Fernández et al., 2015). Briefly, 20 μL of juice/beverage samples (diluted 1:3 with water) was added to 180 μL of radical DPPH (600 μM in methanol) in a 96-well microplate. The absorbance values at 515 nm were detected at the start time (when the sample was added) and 60 min by a plate reader at 25 °C. The FRAP assay was performed according to a method described previously (Yang, Kortesniemi, Yang, & Zheng, 2018). In brief, 20 μL of juice/beverage samples (diluted 1:50 with water) and 180 μL of diluted FRAP reagent were mixed in a 96-well microplate. The reaction was undertaken at 37 °C for 40 min, and the absorbance values were measured at 593 nm against the blank (water). All results were expressed as mg of gallic acid equivalents (GAE)/mL of juice and beverage samples.

2.9. Sensory evaluation of fermented beverages

Sensory characteristics of the strawberry fermented beverages were evaluated by using a generic descriptive analysis. Ten panelists (7 females, 3 males, age 24–55) were trained according to the ISO 8586-1 standard to evaluate the six beverage samples. The three sessions of training and test were completed in one month. The selection of the descriptors was based on previous investigations (Wirth et al., 2012; Kårlund et al., Hanhineva, Lehtonen, Karjalainen, & Sandell, 2015). Descriptors, definitions and reference standards used in the sensory descriptive analysis of strawberry beverages are shown in Table A.1. The intensities of the attributes were rated on a continuous scale, from 0 (none) to 10 (very strong), with the help of anchored reference samples. Prior to sensory analysis, according to the small deviation of chemical data between the two biological replicates, the beverage samples from duplicate fermentations were mixed to reduce the influence of processing variability on the sensory evaluation. The mixed beverages were divided into aliquots of 10 mL in ISO wine tasting glasses (ISO 3591–1977) at room temperature covered with glass lids. The samples were evaluated in triplicate in separate sessions as coded with three-digit numbers and in randomized order. The data were collected using Compusense Cloud software (version 5.6, Guelph, Canada).

2.10. Statistical analysis

One-way ANOVA together with Tukey's HSD was used to compare chemical variances of different cultivars and beverages, and the independent samples *t*-test was used to determine the differences between the averages of strawberry juice and fermented beverage and between two yeast strains using IBM SPSS 26 (SPSS, Inc., Chicago, IL). Three-way ANOVA was applied for sensory results with fermented beverages as fixed factors and sessions and panelists as random factors. Differences reaching a minimum confidence level of 95% were considered statistically significant.

Unsupervised classification with principal component analysis (PCA) was performed to investigate variations in the compositional profiles (n = 28) of strawberry juices (n = 6, 3 cultivars × 2 replicates), fermented beverages (n = 12, 3 cultivars × 2 yeasts × 2 replicates), and in sensory profiles (n = 19) of fermented beverages. Partial least-squares (PLS) regression was used to investigate relationships between the compositional variables (X-data, n = 50) and antioxidant activities (Y-data, n = 2) in the fermented beverages. Multivariate models were performed by using Unscrambler software (version 11, CAMO Software, Oslo, Norway).

3. Results and discussion

3.1. Fermentation kinetics

The fermentation kinetics of strawberry juices from three cultivars with two yeast strains are shown in Fig. 1. The data represent the mean value ± standard deviation (SD) of duplicate fermentations. The fermentation kinetics were different, mainly depending on the strawberry cultivars, while yeast species had a smaller effect. Although different yeast species were used, the fermentative capabilities of the same cultivar samples were similar. All fermentation was completed in 7 days. It is worth noting that the TD strain showed a better fermentative

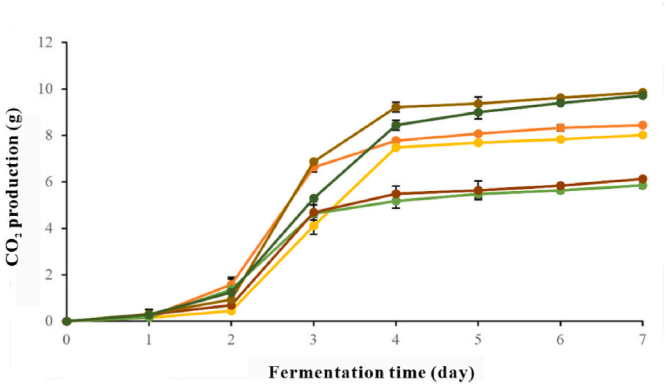

Fig. 1. Fermentation kinetics (mean ± SD, explained by CO_2 production) of strawberry beverages produced by fermentations with *S. cerevisiae* and *T. delbrueckii* (PT PS HT HS LT LS). Abbreviations of the beverage samples refer to Table 1.

performance than the SC strain in the 'Polka' juice, as indicated by a faster fermentation rate. Once the fermentation was terminated, the 'Honeoye' juice released the lowest amount of CO_2 (mean 6.0 g), while the highest quantity of CO_2 was produced in 'Lumotar' juice (mean 9.8 g of CO_2).

3.2. Physicochemical characteristics of fermented beverages

Table 1 illustrates the physicochemical characteristics of strawberry fermented beverages, including pH, °Brix, ethanol content, color properties, sugars, and organic acids. Based on the data in this manuscript, cultivar was the most important factor affecting the physicochemical characteristics of fermented beverages, while the yeasts had a smaller effect. 'Lumotar' beverages (LS and LT) had higher °Brix, stronger yellow and weaker red shade than the others. The highest color density and blue shade in beverages were found in 'Honeoye'.

The average concentration of ethanol was 2.2% and 2.3% in the beverages fermented by SC and TD, respectively. The lowest ethanol content (1.5%) was detected in the 'Honeoye' beverages, which may be because 'Honeoye' juice contained the lowest content of total sugars (37.6 g/L, data not shown). No significant difference was found in the ethanol levels between the beverages produced by TD and SC. The results were in disagreement with a previous report in bilberry wine of lower ethanol yields of TD than SC (Liu et al., 2018). The reason may be that the sugar content in strawberry juices is relatively low, and both TD and SC can completely consume the sugars of juices. However, TD have a very low tolerance to ethanol and other wine components and may not be able to completely convert fermentable sugars during winemaking (Canonico et al., 2016).

Glucose was the only sugar detected in the fermented beverages. All beverages contained low levels of total sugars (0.1 g/L) and sugar/acid ratios (0.02). This proved that the TD strain alone could complete the consumption of the low sugar contents in the starting material. Citric acid was the major organic acid in fermented beverages, followed by malic and ascorbic acids. Among cultivars, the content of total acids was lowest in 'Polka', with nondetected ascorbic acid. Fermentation involving the TD strain consumed more initial citric acid in strawberry juice than fermentations involving the SC strain (55.5 vs 43.4%, respectively). The reduction in individual acids showed little impact on pH values in strawberry beverages.

3.3. Anthocyanins, free ellagic acid and ellagitannins

Seven anthocyanins (peaks 1–7, Fig. A.$_1$) were identified as

Table 1
Physicochemical characteristics of strawberry original juice and fermented beverages[1].

	Comparison of fermented beverages						Fermented beverages by cultivars			Fermented beverages by yeasts		Comparison of original juices and fermented beverages	
	PS[2]	PT	HS	HT	LS	LT	Polka	Honeoye	Lumotar	SC[2]	TD	Juices	Beverages
pH	3.5 ± 0.0^{b}	3.6 ± 0.0^{c}	3.4 ± 0.0^{ab}	3.3 ± 0.0^{a}	3.6 ± 0.1^{c}	3.7 ± 0.0^{c}	3.6 ± 0.0^{b}	3.3 ± 0.0^{a}	3.6 ± 0.0^{b}	3.5 ± 0.1	3.5 ± 0.2	3.4 ± 0.2	3.5 ± 0.2
°Brix	3.7 ± 0.0^{a}	3.7 ± 0.1^{a}	3.7 ± 0.0^{a}	3.7 ± 0.0^{a}	4.2 ± 0.0^{b}	4.4 ± 0.1^{b}	3.6 ± 0.1^{a}	3.7 ± 0.0^{a}	4.3 ± 0.1^{b}	3.8 ± 0.3	3.9 ± 0.4	8.6 ± 1.2^{A}	3.9 ± 0.3^{B}
Color density	2.0 ± 0.1^{b}	1.8 ± 0.1^{ab}	5.1 ± 0.2^{d}	3.6 ± 0.0^{c}	1.4 ± 0.1^{a}	1.5 ± 0.1^{a}	1.9 ± 0.1^{a}	4.4 ± 0.8^{b}	1.5 ± 0.1^{a}	2.8 ± 1.4	2.3 ± 1.0	5.0 ± 1.1^{A}	2.6 ± 1.2^{B}
Hue	0.9 ± 0.0^{c}	1.0 ± 0.0^{d}	0.9 ± 0.0^{b}	0.8 ± 0.0^{a}	1.3 ± 0.0^{f}	1.2 ± 0.0^{e}	0.9 ± 0.0^{b}	0.8 ± 0.0^{a}	1.2 ± 0.1^{c}	1.0 ± 0.2	1.0 ± 0.2	0.7 ± 0.1^{A}	1.0 ± 0.2^{B}
%Yellow	46.9 ± 0.0^{c}	47.2 ± 0.0^{d}	38.6 ± 0.0^{b}	42.8 ± 0.0^{a}	54.4 ± 0.0^{f}	52.6 ± 0.0^{e}	47.1 ± 0.1^{b}	40.7 ± 0.0^{a}	53.5 ± 0.0^{c}	46.6 ± 0.1	47.6 ± 0.0	41.1 ± 0.0^{A}	47.1 ± 0.0^{B}
%Red	50.3 ± 0.0^{d}	49.7 ± 0.0^{c}	44.9 ± 0.0^{e}	54.4 ± 0.0^{f}	41.9 ± 0.0^{a}	44.2 ± 0.0^{b}	50.0 ± 0.0^{b}	49.7 ± 0.1^{b}	43.0 ± 0.0^{a}	45.7 ± 0.0	49.4 ± 0.0	57.0 ± 0.0^{A}	47.6 ± 0.0^{B}
%Blue	2.8 ± 0.0^{a}	3.0 ± 0.0^{a}	16.5 ± 0.0^{b}	2.7 ± 0.0^{a}	3.7 ± 0.0^{a}	3.2 ± 0.0^{a}	2.9 ± 0.0	9.6 ± 0.1	3.4 ± 0.0	7.6 ± 0.1	3.0 ± 0.0	1.9 ± 0.0	5.3 ± 0.1
Ethanol (%, v/v)	2.7 ± 0.1^{b}	2.6 ± 0.2^{b}	1.3 ± 0.3^{a}	1.6 ± 0.4^{a}	2.4 ± 0.4^{b}	2.6 ± 0.8^{b}	2.7 ± 0.1^{b}	1.5 ± 0.4^{a}	2.5 ± 0.5^{b}	2.2 ± 0.6	2.3 ± 0.7	0.0 ± 0.0^{A}	2.2 ± 0.5^{B}
Sugars (g/L)													
Fructose	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	21.2 ± 5.5^{A}	N.D^{B}
Glucose	0.1 ± 0.0^{c}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{a}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{ab}	0.1 ± 0.0	0.1 ± 0.0	30.7 ± 8.6^{A}	0.1 ± 0.0^{B}
Sucrose	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	2.5 ± 0.8^{A}	N.D^{B}
Total sugars[3]	0.1 ± 0.0^{c}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{a}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{a}	0.1 ± 0.0^{ab}	0.1 ± 0.0	0.1 ± 0.0	54.4 ± 13.5^{A}	0.1 ± 0.0^{B}
Organic acids (g/L)													
Malic acid	1.7 ± 0.0^{a}	1.6 ± 0.0^{a}	2.5 ± 0.1^{b}	2.4 ± 0.0^{b}	2.8 ± 0.0^{b}	3.1 ± 0.1^{d}	1.7 ± 0.1^{a}	2.5 ± 0.1^{b}	3.0 ± 0.2^{c}	2.4 ± 0.5	2.4 ± 0.7	3.4 ± 0.8^{A}	2.4 ± 0.6^{B}
Citric acid	3.6 ± 0.0^{b}	3.4 ± 0.1^{b}	5.0 ± 0.3^{c}	4.8 ± 0.0^{c}	5.4 ± 0.1^{d}	5.5 ± 0.1^{a}	3.5 ± 0.1	4.9 ± 0.2	4.2 ± 1.5	4.7 ± 0.9	3.7 ± 0.9	8.3 ± 1.9^{A}	4.2 ± 1.0^{B}
Ascorbic acid	N.D^{a}	N.D^{a}	0.2 ± 0.0^{c}	0.2 ± 0.0^{c}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	N.D^{a}	0.2 ± 0.0^{c}	0.1 ± 0.0^{b}	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Total acids[3]	5.3 ± 0.0^{a}	5.0 ± 0.1^{a}	7.6 ± 0.4^{c}	7.4 ± 0.1^{c}	8.3 ± 0.1^{d}	6.1 ± 0.1^{b}	5.2 ± 0.2^{a}	7.5 ± 0.3^{b}	7.2 ± 1.3^{b}	7.1 ± 1.4	6.2 ± 1.0	11.8 ± 1.7^{A}	6.6 ± 1.3^{B}
Sugar/acid ratio[4]	0.03 ± 0.0^{d}	0.02 ± 0.0^{c}	0.01 ± 0.0^{a}	0.01 ± 0.0^{ab}	0.01 ± 0.0^{b}	0.01 ± 0.0^{c}	0.02 ± 0.0^{b}	0.01 ± 0.0^{a}	0.02 ± 0.0^{a}	0.02 ± 0.0	0.02 ± 0.0	4.81 ± 1.7^{A}	0.02 ± 0.0^{B}

[1] Results represent the mean ± SD. Original juices were analyzed in duplicate and fermented beverages in quadruplicate. Values in the same raw with different superscript letters are considered significantly different at $p < 0.05$, when separately comparing beverages, cultivars, yeasts, or drinks (original juices vs. fermented beverages).

[2] PS/PT, 'Polka' beverages fermented with *S. cerevisiae/T. delbrueckii*, HS/HT, 'Honeoye' beverages fermented with *S. cerevisiae/T. delbrueckii,* LS/LT, 'Lumotar' beverages fermented with *S. cerevisiae/T. delbrueckii*. SC, *S. cerevisiae*; TD, *T. delbrueckii*.

[3] Total sugars = sum of each sugar; total acids = sum of each organic acid.

[4] Sugar/acid ratio = total sugars/total organic acids.

glycosides of cyanidin and pelargonidin, while no free anthocyanin aglycones were detected. Peaks 1 (cyanidin-3-*O*-glucoside, cy-3-glu) and 3 (pelargonidin-3-*O*-glucoside, pe-3-glu) were identified by comparison with the corresponding standards. Four subsequent peaks (2, 4, 6 and 7) showed identical fragmentation to the aglycone at *m/z* 271 and were identified as pelargonidin derivatives (Fig. A.$_{1C}$). Peak 2 had the $[M + H]^+$ ion at *m/z* 595 (271 + 162+162), losing di-hexose and hence was identified as pelargonidin-3,5-diglucoside (pe-3,5-diglu) (Cerezo, Cuevas, Winterhalter, Garcia-Parrilla, & Troncoso, 2010). Peak 4 with $[M + H]^+$ at *m/z* 579 (271 + 162+146) and a fragment ion at *m/z* 271 was identified as pelargonidin-3-*O*-rutinoside (pe-3-rut). Peak 6 with the $[M + H]^+$ ion at *m/z* 519 and a fragment ion at *m/z* 271 lost a sugar component, malonyl-hexose, was identified as pelargonidin-3-*O*-(6″-malonyl)glucoside (pe-3-malonylglu) based on published information (Aaby, Mazur, Nes, & Skrede, 2012; Nowicka, Kucharska, Sokół-Łętowska, & Fecka, 2019). Similar to peak 6, peak 5 was identified as cyanidin-3-*O*-(6″-malonyl)glucoside (cy-3-malonylglu) based on previous studies (Cerezo et al., 2010; Nowicka et al., 2019). The remaining peak 7 was tentatively identified as a pelargonidin derivative with fragment ions at *m/z* 271, but further information was not available to allow speculation about the molecular weight of the compound.

The contents of seven anthocyanins, free ellagic acid and ellagitannins in fermented beverages are summarized in Table 2. Among the six fermented beverages, the total content of anthocyanins ranged from 187.8 (LS) to 287.9 (HT) mg/L, and this difference may be very important, taking into account the impact of anthocyanin content on the antioxidant capacity (Cerezo et al., 2010). As reported previously (Aaby et al., 2012; Nowicka et al., 2019), pe-3-glu was the most abundant anthocyanin in strawberries. Its content varied from 35.8 (LS) to 123.4 (HT) mg/L between all produced beverages ($p < 0.05$), contributing 20–43% of the total anthocyanins in the beverages.

Among the beverages of the three cultivars, the pe-3-glu content in 'Honeoye' beverages was higher than that of the other cultivars. Moreover, the 'Polka' beverages presented the most abundant cy-3-glu, pe-3-malonylglu and cy-3-malonylglu contents, whereas 'Lumotar' had lower contents of the latter two than the other cultivars ($p < 0.05$). Compared

Table 2
The contents of anthocyanins, ellagitannins (mg/L), and total phenolic content and *in vitro* antioxidant capacities (mg gallic acid equivalents/ml) in the strawberry original juice and fermented beverages.[1]

Compounds[2]	cy-3-glu	pe-3,5-diglu	pe-3-glu	pe-3-rut	cy-3-malonyl glu	pe-3-malonylglu	pe derivative	Total ANTs	pe/ total ANTS	cy/ total ANTS	Free EA	ETs	TPC	DPPH	FRAP
Comparison of fermented beverages [3]															
PS	24.4 ± 1.7^{bc}	21.1 ± 1.1	44.9 ± 3.0^{b}	28.8 ± 1.8	27.9 ± 1.1^{b}	50.6 ± 1.4^{c}	23.6 ± 1.3^{a}	221.4 ± 11.5^{b}	0.76 ± 0.0^{a}	0.24 ± 0.0^{d}	23.2 ± 0.5^{c}	83.1 ± 2.6^{b}	0.7 ± 0.1^{b}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
PT	26.6 ± 0.4^{c}	21.4 ± 0.4	64.0 ± 0.8^{c}	30.0 ± 0.1	27.1 ± 0.4^{b}	51.3 ± 0.2^{c}	22.4 ± 0.1^{a}	242.7 ± 1.2^{c}	0.78 ± 0.0^{b}	0.22 ± 0.0^{c}	21.0 ± 0.3^{b}	78.8 ± 2.2^{b}	0.6 ± 0.0^{b}	0.4 ± 0.0^{bc}	0.3 ± 0.0^{a}
HS	21.6 ± 0.0^{ab}	22.4 ± 0.0	71.3 ± 0.7^{d}	27.4 ± 0.2	23.2 ± 0.4^{a}	44.3 ± 0.3^{b}	27.7 ± 0.0^{b}	237.8 ± 1.1^{bc}	0.81 ± 0.0^{c}	0.19 ± 0.0^{b}	15.9 ± 1.4^{a}	56.0 ± 8.4^{a}	1.0 ± 0.0^{d}	0.5 ± 0.0^{c}	0.7 ± 0.0^{c}
HT	23.5 ± 0.8^{b}	22.7 ± 0.6	123.4 ± 0.1^{f}	27.7 ± 0.3	23.7 ± 0.7^{a}	42.9 ± 0.5^{b}	24.0 ± 0.2^{a}	287.9 ± 0.3^{d}	0.84 ± 0.0^{d}	0.16 ± 0.0^{a}	14.7 ± 0.1^{a}	49.3 ± 3.6^{a}	0.8 ± 0.0^{c}	0.4 ± 0.0^{bc}	0.6 ± 0.1^{c}
LS	20.4 ± 0.4^{a}	20.4 ± 0.2	35.8 ± 0.3^{a}	29.3 ± 2.6	21.8 ± 0.6^{a}	37.2 ± 0.3^{a}	23.0 ± 0.2^{a}	187.8 ± 4.0^{a}	0.78 ± 0.0^{b}	0.22 ± 0.0^{c}	14.5 ± 0.0^{a}	51.4 ± 4.2^{a}	0.5 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{a}
LT	22.6 ± 0.4^{ab}	21.6 ± 0.5	79.1 ± 1.0^{e}	30.6 ± 1.3	21.0 ± 0.9^{a}	38.5 ± 0.5^{a}	22.3 ± 0.3^{a}	235.6 ± 1.2^{bc}	0.82 ± 0.0^{c}	0.18 ± 0.0^{b}	14.8 ± 0.2^{a}	50.2 ± 2.7^{a}	0.5 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
Fermented beverages by cultivars															
Polka	25.5 ± 1.6^{b}	21.2 ± 0.7^{a}	54.4 ± 11.2	29.4 ± 1.2	27.5 ± 0.8^{c}	51.0 ± 0.9^{c}	23.0 ± 1.0^{a}	232.0 ± 14.0^{ab}	0.77 ± 0.0^{a}	0.23 ± 0.0^{b}	22.1 ± 1.3^{b}	80.9 ± 3.2^{b}	0.9 ± 0.0^{b}	0.5 ± 0.0^{b}	0.8 ± 0.0^{b}
Honeoye	22.5 ± 1.2^{a}	22.6 ± 0.4^{b}	97.4 ± 30.1	27.5 ± 0.3	23.4 ± 0.5^{b}	43.6 ± 0.9^{b}	25.8 ± 2.2^{b}	262.8 ± 28.9^{b}	0.82 ± 0.0^{b}	0.18 ± 0.0^{a}	15.3 ± 1.1^{a}	52.6 ± 6.6^{a}	1.0 ± 0.0^{b}	0.6 ± 0.0^{b}	1.0 ± 0.0^{c}
Lumotar	21.5 ± 1.3^{a}	21.0 ± 0.7^{a}	57.4 ± 25.0	30.0 ± 1.9	21.4 ± 0.8^{a}	37.8 ± 0.8^{a}	22.6 ± 0.4^{a}	211.8 ± 27.7^{a}	0.80 ± 0.0^{ab}	0.20 ± 0.0^{ab}	14.6 ± 0.2^{a}	50.8 ± 3.0^{a}	0.6 ± 0.1^{a}	0.3 ± 0.0^{a}	0.4 ± 0.1^{a}
Fermented beverages by yeasts															
SC	22.1 ± 2.0	21.3 ± 1.0	50.7 ± 16.6^{X}	28.5 ± 1.7	24.3 ± 2.9	44.0 ± 6.0	24.8 ± 2.4	215.7 ± 23.4^{X}	0.78 ± 0.0	0.22 ± 0.0	17.9 ± 4.2	63.5 ± 15.9	0.7 ± 0.2	0.4 ± 0.1	0.5 ± 0.2
TD	24.2 ± 1.9	21.9 ± 0.8	88.8 ± 27.6^{Y}	29.4 ± 1.5	23.9 ± 2.8	44.2 ± 5.8	22.9 ± 0.8	255.4 ± 25.3^{Y}	0.81 ± 0.0	0.19 ± 0.0	16.8 ± 3.2	59.4 ± 15.2	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
Comparison of original juices and fermented beverages															
Juices	34.6 ± 10.2^{A}	N.D^{A}	266.6 ± 30.9^{A}	31.5 ± 1.0^{A}	24.5 ± 3.8	64.8 ± 12.5^{A}	N.D^{A}	422.1 ± 31.5^{A}	0.86 ± 0.0^{A}	0.14 ± 0.0^{A}	14.9 ± 5.6	61.5 ± 6.6	0.8 ± 0.2	0.5 ± 0.1^{A}	0.7 ± 0.2^{A}
Beverages	23.2 ± 2.2^{B}	21.6 ± 0.9^{B}	69.8 ± 29.5^{B}	29.0 ± 1.6^{B}	24.1 ± 2.7	44.2 ± 5.7^{B}	23.8 ± 2.0^{B}	235.5 ± 31.2^{B}	0.80 ± 0.0^{B}	0.20 ± 0.0^{B}	17.4 ± 3.6	61.5 ± 15.0	0.7 ± 0.2	0.4 ± 0.1^{B}	0.5 ± 0.2^{B}

[1] Results represent the mean ± SD. Original juices were analyzed in duplicate, fermented beverages in quadruplicate, and TPC, DPPH and FRAP in triplicate. Values in the same column with different superscript letters are considered significantly different at $p < 0.05$.

[2] Cyanidin, cy; glucose, glu; pelargonidin, pe; rutinose, rut; anthocyanins, ANTs; ellagic acid, EA; ellagitannins, ETs; Total phenolic content, TPC; DPPH radical scavenging capacity, DPPH; ferric reducing antioxidant power, FRAP.

[3] Abbreviations of the beverages and yeasts refer to Table 1.

to the SC yeast, the beverages fermented by TD yeast contained higher contents of pe-3-glu and total anthocyanins ($p < 0.05$), which also agreed with a previous report showing that the color of drinks can be enhanced by using *T. delbrueckii* yeast during grape fermentation (Minnaar, Ntushelo, Ngqumba, Van Breda, & Jolly, 2015).

Free ellagic acid levels ranged from 14.5 to 23.2 mg/L in all strawberry beverages (Table 2). The concentrations of free EA in the current study were similar to those of strawberries grown in Poland (~15 mg/L) (Oszmiański & Wojdyło, 2009). After acid hydrolysis, two individual compounds with similar UV–vis spectra were taken into account in the quantification. The compounds were both considered conversion products of ETs representing EA (peak 8) and its derivative (peak 9) (Fig. A.$_{1B}$). The content of ETs varied significantly, from 49.3 to 83.1 mg/L in the beverages. The 'Polka' beverages had higher contents of free EA and ETs than the other cultivars; moreover, among the fermented beverages, 'Polka' juice fermentation involving SC (PS) contained the most abundant free EA and ETs ($p < 0.05$). No significant difference was observed in the contents of free EA and ETs between the two yeasts (Table 2).

A PCA model was applied to get more detailed information on the differences and similarities among the fermented beverage samples (Fig. 2A). The first two validated principal components (PCs) explained 72% of the total variance in the data (n = 28). All the variables on the PC-1 correlated with certain cultivars. The beverages of 'Polka' were located on the right side and associated with pe-3-malonylglu, cy-3-malonylglu and ellagitannins. Whereas the beverages of 'Honeoye' were located on the opposite side, which correlated positively with total intensity, pe-3-glu, pe-3,5-diglu and total ANTs. Some components, such as °Brix and hue, were located in the middle of the model and correlated with the beverages of 'Lumotar'.

3.4. Comparison of physicochemical compositions between strawberry juices and fermented beverages

An independent samples *t*-test was used to compare the physicochemical compositions of the averaged values of strawberry juices and fermented beverages (Tables 1 and 2). The pH was almost stable before and after each fermentation, but the °Brix values of fermented beverages decreased significantly compared to the initial °Brix of the juice. Fermentation reduced the color density and redness but increased the yellow component ($p < 0.05$, Table 1). This might be due to anthocyanin reacting with other phenolic compounds, resulting in complex formation and leading to color changes (Klopotek, Otto, & Böhm, 2005). After fermentation, the total sugar contents significantly decreased from 54.4 g/L in juices to <1 g/L in beverages. Moreover, microbial activities consumed citric (~50%) and malic acids (~30%) in the original juices. Changes in organic acid content may have a large influence on the sensory qualities of fermented products (Bressani, Martinez, Sarmento, Borém, & Schwan, 2020). However, fermentation had no significant effect on the content of ascorbic acid. In addition, although the content of total organic acids was reduced by 50% during fermentation, the sugar/acid ratio of the beverage dropped sharply to 0.02 due to the consumption of sugars.

Two anthocyanins, pe-3,5-diglu and pe-derivative, were formed in

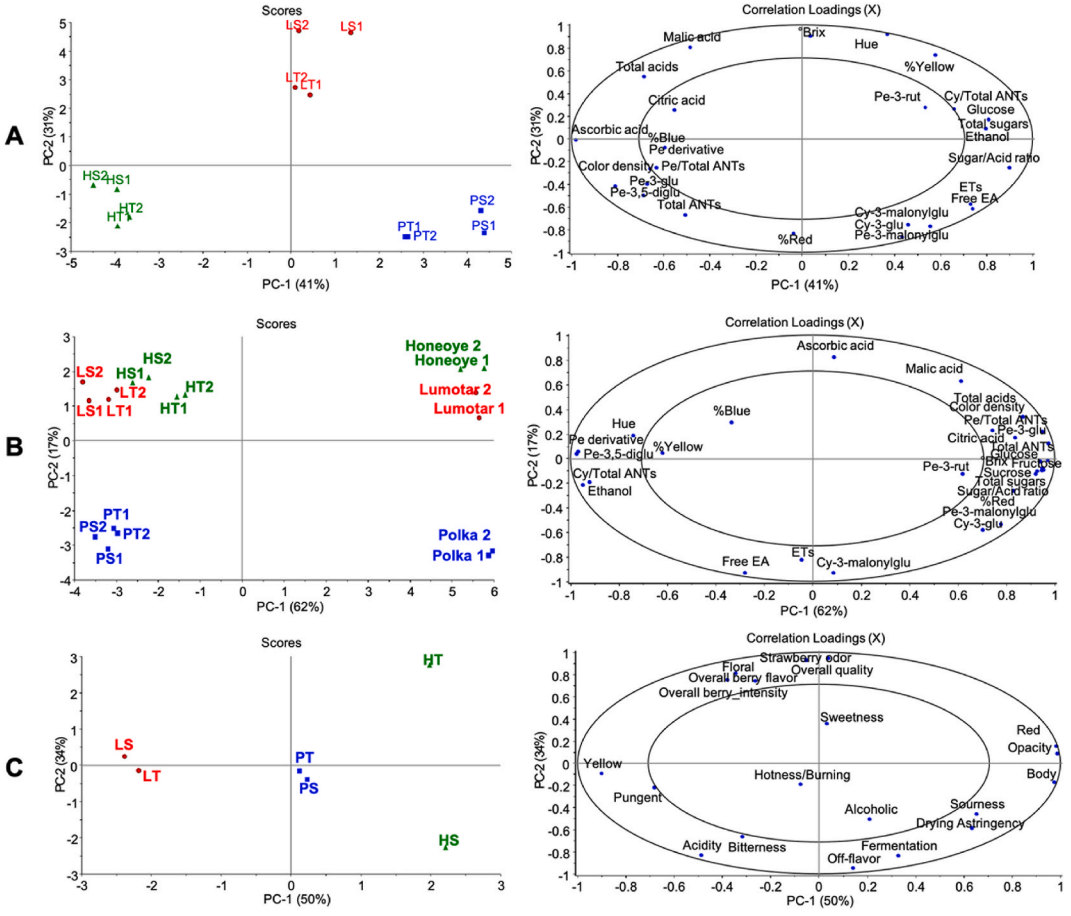

Fig. 2. Principal component analysis models for chemical variables (n = 28), (A) in strawberry fermented beverages (n = 12), (B) comparation between strawberry juices (n = 6) and fermented beverages, (C) for sensory variables (n = 19) in strawberry fermented beverages. Cultivar names are used to indicate the juice samples of cultivars. Cyanidin, cy; glucose, glu; pelargonidin, pe; rutinose, rut; anthocyanins, ANTs; ellagic acid, EA; ellagitannins, ETs. Abbreviations of the beverage samples refer to Table 1.

all fermented beverages as a consequence of the fermentation process (Table 2). Compared to the juice samples, the contents of pelargonidin derivatives were reduced in all beverage samples, especially pe-3-glu and pe-3-malonylglu. The change in profile will affect the bioavailability of strawberry beverages because pe-3-glu is more readily absorbed than other anthocyanins that have been investigated (Mullen, Edwards, Serafini, & Crozier, 2008). Fermentation significantly reduced the total content of anthocyanins from 422.1 mg/L in juices to 235.5 mg/L in beverages. The loss of total anthocyanins during fermentation can be influenced by multiple factors. During fermentation, anthocyanins may interact with other flavonoids, forming more stable pyranoanthocyanins, and the decrease in polarity of these compounds is accompanied by a decrease in solubility (Benito, Morata, Palomero, González, & Suárez-Lepe, 2011). Moreover, yeasts may adsorb anthocyanins in their cell walls, which may cause a reduction in anthocyanins in the final products of alcoholic fermentation (Božič et al., 2020). The fermented beverages contained a little higher content of free EA than the juice beverages, but the difference was not significant. There was no significant effect of fermentation on the content of ETs.

A PCA model was used to study the compositional difference between strawberry juice and fermented beverages (Fig. 2B). The first two validated principal components (PCs) explained 79% of the total variance in the data. The first component showed a clear classification of juices on the right side with sugars, certain anthocyanins (e.g., pe-3-glu), and higher color density. All fermented beverages were located on the left side and associated with pe derivative, pe-3,5-diglu and ethanol, which were only detected in the fermented beverage samples,as well as hue and cy/total ANTs. Some components, such as ascorbic acid and ETs, were located in the middle of the model and showed little correlation with fermentation.

3.5. Volatile compounds

Thirty volatile compounds were semiquantified in strawberry samples (Table A.2). The volatile profile of strawberry samples was dominated by compounds already described (Ulrich, Kecke, & Olbricht, 2018), mainly several esters and alcohols, among which methyl butanoate and ethyl butanoate were reported as key flavor compounds in the typical strawberry-like odor of the juice (Yan et al., 2018). 2,5-Dimethyl-4-methoxy-3(2*H*)-furanone (DMMF), a flavor-impacting compound in strawberry (Yan et al., 2018), is generally described as a sweet aroma (Kallio, 2018). 'Polka' juice showed a higher content of total esters (3631 vs. 1212 and 1442 μg/kg, Table A.2), which led to the highest overall volatile content. Among the beverages, 'Honeoye' juice fermentation involving SC (HS) presented the highest content of total esters (10124 μg/kg) and total volatiles (13866 μg/kg). Compared to the TD yeast, the beverages produced by fermentation involving SC contained 10 to 20 times higher contents of isoamyl acetate ($p < 0.05$), although both yeast strains have been reported to possess a mild ability for acetate formation (Plata, Millan, Mauricio, & Ortega, 2003). The lower production of esters by TD has been reported to correlate with the downregulation of fatty acid biosynthesis (Tondini, Lang, Chen, Herderich, & Jiranek, 2019). Other notable changes in compounds during fermentation were less dependent on the yeast strains.

The contents of ethyl acetate, ethyl hexanoate and isoamyl acetate increased by approximately 10–50 times regardless of the yeast strains after fermentation, while methyl isovalerate and 2-methyl ethyl butanoate, which were present in the original juices, were not detected in the beverages (Table A.2).

3.6. Total phenolic content and in vitro antioxidant capacity

Table 2 shows the results of the total phenolic content (TPC) and antioxidant capacity determined by DPPH and FRAP (mg GAE/mL) in the six strawberry fermented beverages. Generally, 'Honeoye' beverages presented higher TPC and antioxidant capacity than the other cultivars ($p < 0.05$). Among the beverages, 'HS' presented the highest TPC and antioxidant capacity, whereas 'LS' and 'LT' had lower levels for those measurements. In addition, there was no significant difference in these measurements between the two yeasts.

Fermentation did not lead to a significant loss of TPC, probably due to the polymerization and condensation of monomeric phenolics such as anthocyanins (Klopotek et al., 2005). Compared to juice, the DPPH free radical scavenging abilities of beverage samples were significantly decreased (~20%, Table 2). The FRAP results showed a similar trend to the DPPH data and even had a greater degree of decrease (~40%), especially in the 'Polka' samples, e.g., a reduction of ~70% was observed (Table 2). Monomeric anthocyanins mainly contribute to FRAP activity, while polymeric anthocyanins are related to the DPPH scavenging effect (Tsai & Huang, 2004). The initial content of monomeric anthocyanins was heavily decreased during fermentation. Hence, this would account for a substantial part of the heavy reduction in FRAP.

A PLS regression model was created to investigate the contributions of chemical composition (X-variables, n = 50) to the antioxidant capacities (Y-variables, n = 2) of fermented beverages, as shown in Fig. 3. In the model, 67% of the chemical variables explained 94% of the variation in the antioxidant data in the first two factors (validated $R^2 = 0.785$). Factor-1 in Fig. 3 shows major differences among the six beverages based on antioxidant capacities. Total phenolics, pe-3,5-diglu, ascorbic acid, color density and certain aroma volatiles (e.g., linalool) were located on the right side of the correlation loading plot and correlated positively with DPPH and FRAP. Other components, such as ethanol, pH, and pe-3-rut, were on the opposite side and correlated negatively with antioxidant capacity. Linalool has been reported to have similar antioxidative effects to vitamin E (Aprotosoaie, Hăncianu, Costache, & Miron, 2014). Malic and acetic acids, ETs and free EA were located in the middle of the PLS model and showed little correlation with the antioxidant capacities in the fermented beverages. Individual EA acts as a good antioxidant, and the lower contribution to antioxidant activity in the current study might be due to its antagonism with the complex antioxidants in strawberry (Reber, Eggett, & Parker, 2011; Rúa, de Arriaga, García-Armesto, Busto, & Del Valle, 2017).

3.7. Sensory profiles of fermented strawberry beverages

In general, all fermented strawberry beverages presented mainly red color with intense strawberry and floral aroma and were perceived as notably sour and bitter, with a low level of sweetness (Table 3). The low level of sweetness might reduce the overall quality of strawberry fermented beverages because the ideal product of strawberry-flavored beverages should contain higher levels of sweetness (Janiaski, Pimentel, Cruz, & Prudencio, 2016). Moreover, alcoholic content influences the preference for alcoholic drinks, as ethanol concentration increased, the citation of sweetness and fullness/body sensation increased (Ramsey et al., 2018).

Cultivar had an important influence on the appearance of the beverages. When the effects of cultivar were compared, 'Honeoye' beverages were characterized by the highest opacity and intensity of red color, the lowest pungent aroma and the highest sourness. 'Lumotar' beverages were characterized by the highest yellow color shade and the lowest body. The values for 'Polka' were often between the two other cultivars, but its bitterness was higher than others.

Among the individual beverages, 'HT' was rated as having the lowest off-flavor aroma and fermentation odors but the highest berry intensity, strawberry odor and overall quality. 'HS' had the highest astringency, sourness and body intensity, whereas 'LS' had the lowest sourness and body intensity but the highest score for berry flavor. Remarkably, no significant differences were detected in the acidity and sweetness attributes between the beverages.

Compared to SC yeast, higher strawberry odor ($p < 0.05$) and overall quality ($p=0.058$), as well as lower off-flavor and fermentation odor ($p < 0.05$), were observed in the fermentations involving TD.

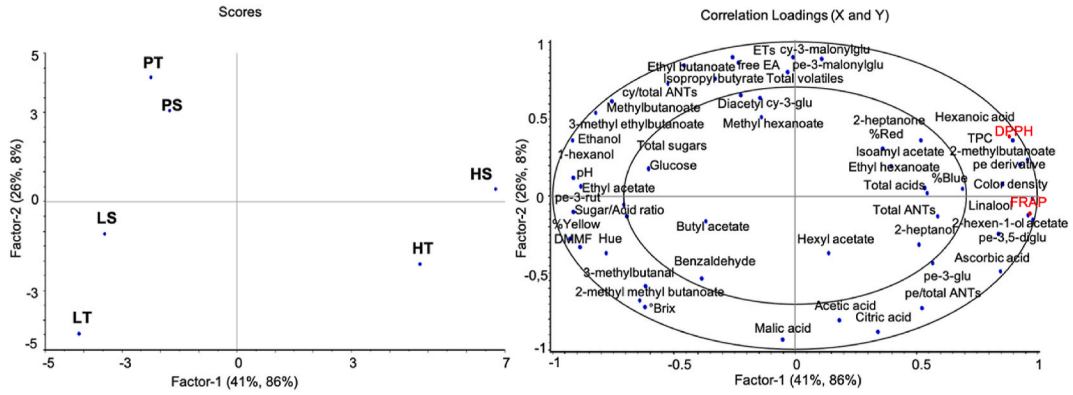

Fig. 3. PLS regression models showing the interactions between (A) chemical variables as X-variables (n = 50, black) and antioxidant abilities as Y-variables (n = 2; red) of strawberry fermented beverages. Cyanidin, cy; glucose, glu; pelargonidin, pe; rutinose, rut; anthocyanins, ANTs; ellagic acid, EA; ellagitannins, ETs; Total phenolic content, TPC; DPPH radical scavenging capacity, DPPH; ferric reducing antioxidant power, FRAP. Abbreviations of the beverage samples refer to Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Sensory attributes and their intensities (scale 0–10) in the strawberry fermented beverages.[1]

	Comparison of fermented beverages[2]						Fermented beverages by cultivars			Fermented beverages by yeasts	
Sensory samples	PS	PT	HS	HT	LS	LT	Polka	Honeoye	Lomutar	SC	TD
Appearance											
Opacity	3.7 ± 2.0^{c}	3.5 ± 2.0^{bc}	4.9 ± 2.9^{d}	5.1 ± 2.3^{d}	2.3 ± 2.2^{a}	2.7 ± 1.9^{ab}	3.6 ± 2.0^{b}	5.0 ± 2.6^{c}	2.5 ± 2.0^{a}	3.6 ± 2.6	3.8 ± 2.3
Red	5.3 ± 2.0^{b}	5.4 ± 2.2^{b}	6.3 ± 2.6^{c}	6.8 ± 2.3^{c}	3.8 ± 2.1^{a}	4.0 ± 2.3^{a}	5.4 ± 2.1^{b}	6.6 ± 2.4^{c}	3.9 ± 2.2^{a}	5.1 ± 2.5	5.4 ± 2.5
Yellow	2.2 ± 1.9^{a}	2.4 ± 2.2^{a}	2.4 ± 2.0^{a}	2.1 ± 1.9^{a}	3.9 ± 2.9^{b}	4.1 ± 2.7^{b}	2.3 ± 2.1^{a}	2.2 ± 2.0^{a}	4.0 ± 2.8^{b}	2.8 ± 2.4	2.9 ± 2.4
Odors											
Overall berry intensity	5.8 ± 1.6^{bc}	5.0 ± 2.3^{ab}	4.8 ± 2.1^{a}	6.4 ± 1.8^{c}	6.0 ± 2.1^{bc}	6.1 ± 2.0^{c}	5.4 ± 2.0	5.6 ± 2.1	6.0 ± 2.0	5.5 ± 2.0	5.9 ± 2.1
Strawberry odor	4.8 ± 1.9^{ab}	4.8 ± 2.13^{ab}	4.0 ± 2.0^{a}	6.2 ± 1.8^{c}	4.8 ± 2.3^{ab}	5.4 ± 2.3^{bc}	4.8 ± 2.0	5.1 ± 2.2	5.1 ± 2.3	4.5 ± 2.1^{X}	5.4 ± 2.1^{Y}
Pungent	2.8 ± 1.9^{ab}	3.3 ± 1.6^{b}	2.3 ± 1.9^{ab}	2.1 ± 1.7^{a}	2.8 ± 1.8^{ab}	3.2 ± 1.8^{b}	3.1 ± 1.8^{b}	2.2 ± 1.8^{a}	3.0 ± 1.8^{b}	2.6 ± 1.9	2.9 ± 1.8
Alcoholic	2.6 ± 2.1^{ab}	3.2 ± 2.1^{b}	2.9 ± 2.1^{ab}	2.4 ± 2.1^{ab}	2.3 ± 1.7^{a}	2.8 ± 1.7^{ab}	2.9 ± 2.1	2.7 ± 2.1	2.5 ± 1.7	2.6 ± 2.0	2.8 ± 2.0
Fermentation	1.6 ± 1.7^{a}	1.1 ± 1.4^{a}	2.4 ± 2.2^{b}	1.0 ± 1.3^{a}	1.4 ± 1.8^{a}	1.3 ± 1.2^{a}	1.3 ± 1.6	1.7 ± 1.9	1.4 ± 1.6	1.8 ± 1.9^{X}	1.1 ± 1.3^{Y}
Acidity	2.4 ± 1.6	2.5 ± 1.5	2.5 ± 1.9	1.8 ± 1.6	2.4 ± 1.7	2.5 ± 2.0	2.4 ± 1.6	2.2 ± 1.8	2.5 ± 1.9	2.4 ± 1.7	2.3 ± 1.7
Floral	3.7 ± 1.7^{ab}	3.8 ± 1.7^{ab}	3.2 ± 1.8^{a}	4.1 ± 1.5^{ab}	4.3 ± 1.4^{b}	3.6 ± 1.8^{ab}	3.8 ± 1.7	3.7 ± 1.7	3.9 ± 1.6	3.7 ± 1.7	3.8 ± 1.6
Off-flavor	2.0 ± 1.9^{b}	1.9 ± 2.2^{b}	3.3 ± 2.8^{c}	0.8 ± 1.2^{a}	2.1 ± 2.1^{b}	1.7 ± 1.5^{ab}	1.9 ± 2.1	2.1 ± 2.5	1.9 ± 1.8	2.4 ± 2.4^{X}	1.4 ± 1.7^{Y}
Palate											
Body	2.5 ± 1.8^{abc}	2.5 ± 1.6^{abc}	2.9 ± 2.1^{c}	2.7 ± 1.9^{bc}	1.9 ± 1.5^{a}	2.0 ± 1.6^{ab}	2.5 ± 1.7^{b}	2.8 ± 2.0^{b}	2.0 ± 1.5^{a}	2.4 ± 1.8	2.4 ± 1.7
Hotness/Burning	1.9 ± 1.5^{a}	2.7 ± 2.0^{ab}	2.7 ± 2.0^{ab}	2.4 ± 2.2^{ab}	2.8 ± 2.4^{b}	2.3 ± 1.6^{ab}	2.3 ± 1.8	2.5 ± 2.1	2.5 ± 2.1	2.4 ± 2.0	2.4 ± 1.9
Flavor											
Overall berry flavor	4.6 ± 1.8^{ab}	5.2 ± 1.8^{bc}	4.2 ± 2.2^{a}	5.4 ± 2.2^{bc}	5.6 ± 1.8^{c}	4.5 ± 1.9^{ab}	4.9 ± 1.8	4.8 ± 2.3	5.0 ± 1.9	4.8 ± 2.0	5.0 ± 2.0
Sourness	5.4 ± 1.6^{a}	5.3 ± 1.7^{a}	6.2 ± 1.6^{b}	5.6 ± 1.6^{ab}	5.1 ± 1.8^{a}	5.6 ± 1.4^{ab}	5.3 ± 1.7^{a}	5.9 ± 1.6^{b}	5.3 ± 1.6^{a}	5.6 ± 1.7	5.5 ± 1.5
Sweetness	1.1 ± 0.7	1.2 ± 1.0	0.9 ± 0.9	1.1 ± 1.2	1.2 ± 0.7	0.8 ± 0.8	1.1 ± 0.9	1.0 ± 1.0	1.0 ± 0.7	1.0 ± 0.8	1.0 ± 1.0
Bitterness	6.1 ± 1.9^{c}	5.4 ± 2.2^{bc}	5.5 ± 2.6^{bc}	4.5 ± 2.1^{a}	5.1 ± 2.3^{ab}	5.9 ± 2.0^{bc}	5.8 ± 2.1^{b}	5.0 ± 2.4^{a}	5.5 ± 2.2^{ab}	5.5 ± 2.3	5.3 ± 2.2
Drying Astringency	5.1 ± 2.0^{ab}	5.3 ± 1.9^{ab}	5.6 ± 2.3^{b}	4.8 ± 2.0^{ab}	4.9 ± 2.2^{ab}	4.5 ± 2.4^{a}	5.2 ± 1.9	5.2 ± 2.2	4.7 ± 2.3	5.2 ± 2.2	4.9 ± 2.1
Overall quality	3.8 ± 1.9^{ab}	4.3 ± 2.1^{b}	3.3 ± 2.1^{a}	5.3 ± 2.0^{c}	4.5 ± 1.9^{bc}	3.8 ± 1.8^{ab}	4.0 ± 2.0	4.3 ± 2.3	4.1 ± 1.9	3.9 ± 2.0	4.4 ± 2.0

[1] Results represent the mean ± SD. Values in the same raw with different superscript letters are considered significantly different at $p < 0.05$, when separately comparing fermented beverages, cultivars and yeasts. The explanations of sensory attributes refer Table A.1.

[2] Abbreviations of the fermented beverages and yeasts refer to Table 1.

A PCA model was created to further investigate the sensory profile (n = 19) of fermented beverages (Fig. 2C). The first two validated principal components (PCs) explained 84% of the sensory variance in the model. The attributes of sourness, body and drying astringency, as well as the appearances of red and opacity located on the right part of the loading plot with 'Honeoye' samples (HS and HT). 'Lumotar' samples (LT and LS) had obvious yellow appearance, higher overall berry intensity, and stronger pungent odor. 'Polka' samples (PT and PS) were located in the middle of the loading plot, characterizing the alcoholic attribute, and showed little correlation with other attributes.

4. Conclusion

Among the cultivars, 'Honeoye' beverages presented the highest color density, total anthocyanins and phenolics, and antioxidant capacities but the lowest ethanol content. The beverages fermented with *T. delbrueckii* retained more anthocyanins and strawberry flavor and presented higher overall sensory qualities and lower off-flavor odor than those fermented with *S. cerevisiae.* Although the conclusions in this study were made based on a limited data set, i.e., only one strain of each yeast species, and three strawberry cultivars, as well as each fermentation performed in duplicate, the results have demonstrated that cultivar was more important factor affecting the chemical composition and antioxidant capacity of the fermented beverages than yeasts, and *T. delbrueckii* is the preferred yeast for the fermentation of strawberry juices to produce low-alcohol beverages than the specific SC yeast used in this study. The utilization of *T. delbrueckii* in the production of low-alcohol beverages is a viable alternative method to process surpluses of harvested berries and other underused fruits, resulting in the introduction of novel products into the market. It would be expected that a similar trend may be observed for other strains. However, due to the high genetic variability found among yeast strains within a species, this observation will have to be substantiated with further research.

CRediT authorship contribution statement

Wei Yang: Data curation, Writing – original draft. **Shuxun Liu:** Investigation. **Alexis Marsol-Vall:** Investigation. **Roni Tähti:** Investigation. **Oskar Laaksonen:** Review. **Saila Karhu:** Resources. **Baoru Yang:** Supervision. **Xueying Ma:** Founding, Writing – review & editing.

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.lwt.2021.111910.

References

Aaby, K., Mazur, S., Nes, A., & Skrede, G. (2012). Phenolic compounds in strawberry (*fragaria* × *ananassa* duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chemistry, 132*(1), 86–97. https://doi.org/10.1016/j.foodchem.2011.10.037

Álvarez-Fernández, M. A., Cerezo, A. B., Cañete-Rodríguez, A. M., Troncoso, A. M., & García-Parrilla, M. C. (2015). Composition of nonanthocyanin polyphenols in alcoholic-fermented strawberry products using LC–MS (QTRAP), high-resolution MS (UHPLC-Orbitrap-MS), LC-DAD, and antioxidant activity. *Journal of Agricultural and Food Chemistry, 63*(7), 2041–2051. https://doi.org/10.1021/jf506076n

Álvarez-Fernández, M. A., Hornedo-Ortega, R., Cerezo, A. B., Troncoso, A. M., & García-Parrilla, M. C. (2014). Non-anthocyanin phenolic compounds and antioxidant activity of beverages obtained by gluconic fermentation of strawberry. *Innovative Food Science & Emerging Technologies, 26*, 469–481. https://doi.org/10.1016/j.ifset.2014.08.001

Aprotosoaie, A. C., Hăncianu, M., Costache, I., & Miron, A. (2014). Linalool: A review on a key odorant molecule with valuable biological properties. *Flavour and Fragrance Journal, 29*(4), 193–219. https://doi.org/10.1002/ffj.3197

Benito, S., Morata, A., Palomero, F., González, M., & Suárez-Lepe, J. (2011). Formation of vinylphenolic pyranoanthocyanins by *Saccharomyces cerevisiae* and *pichia guillermondii* in red wines produced following different fermentation strategies. *Food Chemistry, 124*(1), 15–23. https://doi.org/10.1016/j.foodchem.2010.05.096

Božič, J. T., Butinar, L., Albreht, A., Vovk, I., Korte, D., & Vodopivec, B. M. (2020). The impact of *Saccharomyces* and non-*Saccharomyces* yeasts on wine colour: A laboratory study of vinylphenolic pyranoanthocyanin formation and anthocyanin cell wall adsorption. *LWT, 123*, 109072. https://doi.org/10.1016/j.lwt.2020.109072

Bressani, A. P. P., Martinez, S. J., Sarmento, A. B. I., Borém, F. M., & Schwan, R. F. (2020). Organic acids produced during fermentation and sensory perception in specialty coffee using yeast starter culture. *Food Research International, 128*, 108773. https://doi.org/10.1016/j.foodres.2019.108773

Bucher, T., Deroover, K., & Stockley, C. (2018). Low-alcohol wine: A narrative review on consumer perception and behaviour. *Beverages, 4*(4), 82. https://doi.org/10.3390/beverages4040082

Canonico, L., Agarbati, A., Comitini, F., & Ciani, M. (2016). *Torulaspora delbrueckii* in the brewing process: A new approach to enhance bioflavour and to reduce ethanol content. *Food Microbiology, 56*, 45–51. https://doi.org/10.1016/j.fm.2015.12.005

Carew, A. L., Smith, P., Close, D. C., Curtin, C., & Dambergs, R. G. (2013). Yeast effects on pinot noir wine phenolics, color, and tannin composition. *Journal of Agricultural and Food Chemistry, 61*(41), 9892–9898. https://doi.org/10.1021/jf4018806

Cerezo, A. B., Cuevas, E., Winterhalter, P., Garcia-Parrilla, M., & Troncoso, A. (2010). Isolation, identification, and antioxidant activity of anthocyanin compounds in camarosa strawberry. *Food Chemistry, 123*(3), 574–582. https://doi.org/10.1016/j.foodchem.2010.04.073

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough study of reactivity of various compound classes toward the Folin–ciocalteu reagent. *Journal of Agricultural and Food Chemistry, 58*(14), 8139–8144. https://doi.org/10.1021/jf1005935

Ezzat-Zadeh, Z., Henning, S. M., Yang, J., Woo, S. L., Lee, R., Huang, J., et al. (2021). California strawberry consumption increased the abundance of gut microorganisms related to lean body weight, health and longevity in healthy subjects. *Nutrition Research, 85*, 60–70. https://doi.org/10.1016/j.nutres.2020.12.006

FAOSTAT. (2018). *Food and agriculture organization corporate statistical database.* FAO Online Database. Available Online: http://www.Fao.Org/Faostat/En/# Data. (Accessed 23 November 2020).

Giampieri, F., Alvarez-Suarez, J. M., & Battino, M. (2014). Strawberry and human health: Effects beyond antioxidant activity. *Journal of Agricultural and Food Chemistry, 62*(18), 3867–3876. https://doi.org/10.1021/jf405455n

He, W., Liu, S., Heponiemi, P., Heinonen, M., Marsol-Vall, A., Ma, X., et al. (2020). Effect of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains on chemical composition and sensory quality of ciders made from Finnish apple cultivars. *Food Chemistry*. , Article 128833. https://doi.org/10.1016/j.foodchem.2020.128833

Holt, R. R., Zuelch, M. L., Charoenwoodhipong, P., Al-Dashti, Y. A., Hackman, R. M., & Keen, C. L. (2020). Effects of short-term consumption of strawberry powder on select parameters of vascular health in adolescent males. *Food & Function, 11*(1), 32–44. https://doi.org/10.1039/C9FO01844A

Janiaski, D. R., Pimentel, T. C., Cruz, A. G., & Prudencio, S. H. (2016). Strawberry-flavored yogurts and whey beverages: What is the sensory profile of the ideal product? *Journal of Dairy Science, 99*(7), 5273–5283. https://doi.org/10.3168/jds.2015-10097

Kallio, H. P. (2018). Historical review on the identification of mesifurane, 2, 5-dimethyl-4-methoxy-3(2*H*)-Furanone, and its occurrence in berries and fruits. *Journal of Agricultural and Food Chemistry, 66*(11), 2553–2560. https://doi.org/10.1021/acs.jafc.8b00519

Kårlund, A., Hanhineva, K., Lehtonen, M., Karjalainen, R. O., & Sandell, M. (2015). Nontargeted metabolite profiles and sensory properties of strawberry cultivars grown both organically and conventionally. *Journal of Agricultural and Food Chemistry, 63*(3), 1010–1019. https://doi.org/10.1021/jf505183j

Kelanne, N., Laaksonen, O., Seppälä, T., Yang, W., Tuukkanen, K., Loponen, J., et al. (2019). Impact of cyclodextrin treatment on composition and sensory properties of lingonberry (*vaccinium vitis-idaea*) juice. *LWT, 113*, 108295. https://doi.org/10.1016/j.lwt.2019.108295

Klopotek, Y., Otto, K., & Böhm, V. (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 53*(14), 5640–5646. https://doi.org/10.1021/jf047947v

Koponen, J. M., Happonen, A. M., Mattila, P. H., & Törrönen, A. R. (2007). Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *Journal of Agricultural and Food Chemistry, 55*(4), 1612–1619. https://doi.org/10.1021/jf062897a

Liu, S., Laaksonen, O., Kortesniemi, M., Kalpio, M., & Yang, B. (2018). Chemical composition of bilberry wine fermented with non-*Saccharomyces* yeasts (*Torulaspora delbrueckii* and *Schizosaccharomyces pombe*) and *Saccharomyces cerevisiae* in pure, sequential and mixed fermentations. *Food Chemistry, 266*, 262–274. https://doi.org/10.1016/j.foodchem.2018.06.003

Liu, S., Marsol-Vall, A., Laaksonen, O. A., Kortesniemi, M., & Yang, B. (2020). Characterization and quantification of non-anthocyanin phenolic compounds in white and blue bilberry (*vaccinium myrtillus*) juices and wines using UHPLC–DAD–ESI-QTOF-MS. *Journal of Agricultural and Food Chemistry, 68*(29), 7734–7744. https://doi.org/10.1021/acs.jafc.0c02842

Ma, X., Laaksonen, O., Heinonen, J., Sainio, T., Kallio, H., & Yang, B. (2017). Sensory profile of ethyl *β*-D-glucopyranoside and its contribution to quality of sea buckthorn (*hippophaë rhamnoides* L.). *Food Chemistry, 233*, 263–272. https://doi.org/10.1016/j.foodchem.2017.04.073

Minnaar, P., Ntushelo, N., Ngqumba, Z., Van Breda, V., & Jolly, N. (2015). Effect of *Torulaspora delbrueckii* yeast on the anthocyanin and flavanol concentrations of cabernet franc and pinotage wines. *South African Journal of Enology and Viticulture, 36*(1), 50–58.

Mullen, W., Edwards, C. A., Serafini, M., & Crozier, A. (2008). Bioavailability of pelargonidin-3-*O*-glucoside and its metabolites in humans following the ingestion of strawberries with and without cream. *Journal of Agricultural and Food Chemistry, 56*(3), 713–719. https://doi.org/10.1021/jf072000p

Nowicka, A., Kucharska, A. Z., Sokół-Łętowska, A., & Fecka, I. (2019). Comparison of polyphenol content and antioxidant capacity of strawberry fruit from 90 cultivars of Fragaria×Ananassa duch. *Food Chemistry, 270*, 32–46. https://doi.org/10.1016/j.foodchem.2018.07.015

Oszmiański, J., & Wojdyło, A. (2009). Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. *European Food Research and Technology, 228*(4), 623–631. https://doi.org/10.1007/s00217-008-0971-2

Pickering, G. J. (2000). Low-and reduced-alcohol wine: A review. *Journal of Wine Research, 11*(2), 129–144. https://doi.org/10.1080/09571260020001575

Plata, C., Millan, C., Mauricio, J., & Ortega, J. (2003). Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. *Food Microbiology, 20*(2), 217–224. https://doi.org/10.1016/S0740-0020(02)00101-6

Ramsey, I., Ross, C., Ford, R., Fisk, I., Yang, Q., Gomez-Lopez, J., et al. (2018). Using a combined temporal approach to evaluate the influence of ethanol concentration on liking and sensory attributes of lager beer. *Food Quality and Preference, 68*, 292–303. https://doi.org/10.1016/j.foodqual.2018.03.019

Reber, J. D., Eggett, D. L., & Parker, T. L. (2011). Antioxidant capacity interactions and a chemical/structural model of phenolic compounds found in strawberries. *International Journal of Food Sciences and Nutrition, 62*(5), 445–452. https://doi.org/10.3109/09637486.2010.549115

Rúa, J., de Arriaga, D., García-Armesto, M. R., Busto, F., & Del Valle, P. (2017). Binary combinations of natural phenolic compounds with gallic acid or with its alkyl esters: An approach to understand the antioxidant interactions. *European Food Research and Technology, 243*(7), 1211–1217. https://doi.org/10.1007/s00217-016-2838-2

Shi, W., Wang, J., Chen, F., & Zhang, X. (2019). Effect of *issatchenkia terricola* and *pichia kudriavzevii* on wine flavor and quality through simultaneous and sequential Co-fermentation with *Saccharomyces cerevisiae*. *LWT, 116*, 108477. https://doi.org/10.1016/j.lwt.2019.108477

Tondini, F., Lang, T., Chen, L., Herderich, M., & Jiranek, V. (2019). Linking gene expression and oenological traits: Comparison between *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* strains. *International Journal of Food Microbiology, 294*, 42–49. https://doi.org/10.1016/j.ijfoodmicro.2019.01.014

Tsai, P. J., & Huang, H. P. (2004). Effect of polymerization on the antioxidant capacity of anthocyanins in Roselle. *Food Research International, 37*(4), 313–318. https://doi.org/10.1016/j.foodres.2003.12.007

Ulrich, D., Kecke, S., & Olbricht, K. (2018). What do we know about the chemistry of strawberry aroma? *Journal of Agricultural and Food Chemistry, 66*(13), 3291–3301. https://doi.org/10.1021/acs.jafc.8b01115

Voidarou, C., Antoniadou, M., Rozos, G., Tzora, A., Skoufos, I., Varzakas, T., et al. (2021). Fermentative foods: Microbiology, biochemistry, potential human health benefits and public health issues. *Foods, 10*(1), 69. https://doi.org/10.3390/foods10010069

Wirth, J., Caille, S., Souquet, J. M., Samson, A., Dieval, J., Vidal, S., et al. (2012). Impact of post-bottling oxygen exposure on the sensory characteristics and phenolic composition of grenache rosé wines. *Food Chemistry, 132*(4), 1861–1871. https://doi.org/10.1016/j.foodchem.2011.12.019

Yan, J., Ban, Z., Lu, H., Li, D., Poverenov, E., Luo, Z., et al. (2018). The aroma volatile repertoire in strawberry fruit: A review. *Journal of the Science of Food and Agriculture, 98*(12), 4395–4402. https://doi.org/10.1002/jsfa.9039

Yang, W., Kortesniemi, M., Yang, B., & Zheng, J. (2018). Enzymatic acylation of anthocyanins isolated from alpine bearberry (*arctostaphylos alpina*) and lipophilic properties, thermostability, and antioxidant capacity of the derivatives. *Journal of Agricultural and Food Chemistry, 66*(11), 2909–2916. https://doi.org/10.1021/acs.jafc.7b05924