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Comparison of phenolic composition and sensory quality among pear beverages made using *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*

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ABSTRACT

The effects of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* on phenolic composition and sensory quality were characterized in the production of alcoholic beverages from selected pear cultivars with diverse biochemical characteristics. The fermentation process generally affected the phenolic composition by increasing the contents of hydroxycinnamic acids and flavan-3-ols and reducing the levels of hydroxybenzoic acids, procyanidins, and flavonols. Although the phenolic compositions and sensory properties of pear beverages depended primarily on pear cultivar selection, the applied yeast strains also played important roles in beverage quality. Fermentation with *T. delbrueckii* resulted in higher caffeoylquinic acid and quercetin-3-*O*-glucoside contents, higher rated intensities of 'cooked pear' and 'floral' odors and a sweeter taste than fermentation with *S. cerevisiae*. Moreover, higher concentrations of hydroxybenzoic acids, hydroxycinnamic acids, and flavonols correlated closely with astringency perception. Applying *T. delbrueckii* strains and breeding novel pear cultivars are important approaches to produce fermented beverages of high quality.

1. Introduction

Fruit wines are fermented and undistilled alcoholic beverages made from fruit juices (mostly from the juices of grapes or apples). According to the European Cider and Fruit Wine Association, the annual production of fruit wines reached 24.7 million HL in 2021. In Northern European countries, there is growing interests in developing local fruit cultivars as beverage cultivars, especially due to increased fruit production, e.g., in Finland by 30% during the last 5 years (Natural Resources Institute Finland - Statistics service). Fermentation of alcoholic fruit beverages is a comprehensive bioprocessing method that converts macronutrients (e.g., sugars and proteins) into ethanol, carbon dioxide, peptides, and secondary metabolites. Either originally present in the fruits or derived from fermentation processes, these metabolites significantly influence the aroma, taste, and mouth-feel sensations of beverages (He et al., 2021).

Pears (*Pyrus* spp.) are well known for their high nutritional values and health benefits due to their abundance of sugars, amino acids, minerals, fibers, and bioactive components (Kolniak-Ostek, Kłopotowska, Rutkowski, Skorupinska, & Kruczynska, 2020; Sun et al., 2021). The major bioactive compounds present in pear fruits are phenolic compounds, the compositions of which are significantly influenced by biological and environmental factors, such as cultivars, tissues, maturity, light exposure, and storage conditions (Brahem et al., 2017). Large amounts of harvested pome fruits (up to 60%) are lost or wasted during postharvest storage, industrial processing, and household consumption (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018). Thus, processing pears into pear wines or other fermented beverages (alcoholic or nonalcoholic) can reduce fruit loss and provide novel value-added pear products.

The yeasts applied in fermentation also influence the nutritional components and sensory qualities of alcoholic fruit beverages. As the

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most commercially important yeast, *S. cerevisiae* has been widely used in winemaking. *S. cerevisiae* has high tolerance to harsh industrial conditions and is suitable for use in large-scale operations (Lian et al., 2018). The utilization of other yeasts in fruit winemaking has also been investigated in previous studies (Wei, Zhang, Yuan, Dai, & Yue, 2019). Among the non-*Saccharomyces* yeasts, *T. delbrueckii* is the most suitable, commercialized, and widely utilized microbe in industrial wine production (Benito, 2018). *T. delbrueckii* was reported to produce lower contents of acetaldehyde, acetoin, ethanol, and acetate than *S. cerevisiae* (Tondini, Lang, Chen, Herderich, & Jiranek, 2019). Moreover, *T. delbrueckii* was also acknowledged as preventing microorganism spoilage, as well as enzymatic and chemical oxidation during grape winemaking (Simonin, Alexandre, Nikolantonaki, Coelho, & Tourdot-Maréchal, 2018). The sequential inoculation of *T. delbrueckii* and *Saccharomyces* yeast (*S. bayanus* or *S. cerevisiae*) markedly increased the ester contents (mainly ethyl esters, by 53%) and decreased the level of volatile acids (by 33%) in 'Jinchuan' (*Pyrus ussuriensis* Maxim.) pear wines (Liu et al., 2022). Yang and his coworkers (2022) found that the coculture of *S. cerevisiae* and *T. delbrueckii* led to higher contents of ethyl esters (by 15–35%) in 'Zaosu' (*Pyrus pyrifolia* × *Pyrus communis*) pear wines than a single culture of *S. cerevisiae*. However, the utilization of non-*Saccharomyces* yeasts in the fermentation of alcoholic pear beverages has not been systematically studied, especially regarding their impacts on nonvolatile phenolic compounds and the related flavor properties (such as astringency and bitterness). Thus, the approach in our study could constitute an excellent tool for comparing the chemical compositions and sensorial properties of juices and alcoholic beverages made from pears.

The chemical compositions of untreated juices produced from certain unreleased breeding selections and cultivars of European pears were investigated in our previous study (He et al., 2022a), as well as the relationship between the key phenolic variables and the usage grouping developed by breeders. In a previous study, the development of new pear cultivars was assisted by providing chemical targets for breeders (He et al., 2022a). This research is a follow-up study aiming to assist the Finnish beverage industry in the production of high-quality fermented pear beverage products (or 'perry'). In the present study, sensory and chemical profiles from pasteurized juices and their alcoholic beverage products were investigated from pear fruits representing the three usage groups, namely, 'dessert usage group', 'dessert/perry usage group', and 'perry usage group'. Two commercial pear cultivars were selected as external standard cultivars for comparison. Our special focus was on the effects of yeast fermentation and yeast species (*S. cerevisiae* and *T. delbrueckii* strains) on the phenolic compositions and sensory properties of alcoholic pear beverages made from pasteurized single-cultivar juices representing a selected set of diverse types of cultivars. As hypotheses, both cultivar usage group and yeast selections were expected to have significant effects on the composition and sensory quality. The present evaluation of chemical and sensory characteristics provides new avenues to control and tailor pear product qualities with high economic potential.

2. Materials and methods

2.1. Pear cultivars and yeast strains

Pears of seven unreleased breeding selections ('Py2', 'Py4', 'Py7', 'Py10', 'Py11', 'Py12', and 'Py13'; Table 1) from the Finnish pear breeding program of Natural Resources Institute Finland (Luke) were used in this study. Two test pear cultivars ('Stolishnaja' and 'Krupnoplodnaja') originating from Russia were selected from our previous study (He et al., 2022a). All fruit samples were harvested in Piikkiö, Kaarina, Finland (60°39′N, 22°55′E, the experimental orchard of Luke) in 2019. Fruits of two commercial cultivars ('Conference' originating from the United Kingdom and 'Clara Frijs' originating from Denmark) were obtained from a local market in Turku, Finland. The yeast strains *S. cerevisiae* Lalvin V1116 (SC1116) and *T. delbrueckii* 291 (TD291) were obtained from Lallemand Inc. (Montreal, Canada).

Table 1
Description of eleven pear breeding selections and cultivars.

Name of cultivar or cross	Cultivar or breeding	Fruit description[b]	Abbreviations
Pepi × Lück	selection	dessert pear, mild sweetness and mildly aromatic	Py2
Pepi × Lück	selection	dessert pear, sweet and aromatic	Py4[c]
Alna × Lück	selection	dessert/perry pear, juicy, sweet, mildly acidic, and aromatic	Py7
Karmla × Pakurlan Päärynä	selection	perry pear, sweet, astringent, bitter, and spicy	Py10[c]
Rumnaja Kedrina × Pakurlan Päärynä	selection	dessert/perry pear, juicy, mild sweetness, mildly astringent, mildly aromatic	Py11
Rumnaja Kedrina × Pakurlan Päärynä	selection	perry pear, astringent, and mildly bitter	Py12[c]
Lukna × Pakurlan Päärynä	selection	dessert pear, sweet, aromatic, and mildly astringent	Py13
Stolishnaja /Stolichnaya	cultivar	perry pear, acidic, astringent, and juicy	Sto[c]
Krupnoplodnaja Susova	cultivar	dessert pear, juicy	Kru[c]
Conference	cultivar	commercial dessert pear	Con
Clara Frijs	cultivar	commercial dessert pear	Cla[c]

[a] The name of cross is showed with 'maternal cultivar × pollen cultivar'.

[b] The pears were divided into three pear usage groups: 'dessert', 'perry', and 'perry/dessert', which was determined by breeders according to the perceived sweetness, sourness, bitterness, and astringency of the studied pear cultivars and breeding selections ('Py2', 'Py4', 'Py7', 'Py10', 'Py11', 'Py12', 'Py13', 'Sto', and 'Kru'). In addition, the commercial pear cultivars 'Conference' and 'Clara Frijs' were produced as commercial dessert pear cultivars, thus, they were in the 'dessert pear usage group'.

[c] Different pear selections ('Py4', 'Py12', and 'Py10'), two test cultivars ('Stolishnaia' and 'Krupnoplodnaja Susova'), as well as one commercial pear cultivar ('Clara Frijs') were used in the sensory evaluation.

2.2. Alcoholic beverage preparation

All fruits were processed when they reached their eating ripeness as determined by the reduced flesh firmness and increased flesh juiciness and sensory sweetness. Fruits with obvious signs of biological contamination (storage rot or physiological deterioration) were excluded. The fruit sample batches (approx. 2.5–4 kg) were all processed into juices in triplicate batches using a continuous centrifugal juice extractor attached to a food processor (Kenwood, Havant, United Kingdom) and centrifuged (3000 × g, 10 min) to remove the puree to obtain the untreated juices. The chemical compositions of these untreated pear juices have been reported in our previous study (He et al., 2022a). Then, the pear juices were combined and pasteurized at 95 °C for 5 min, leading to inactivation of the enzymes and microbes in the pasteurized juices (Liu, Marsol-Vall, Laaksonen, Kortesniemi, & Yang, 2020). After cooling to room temperature under running tap water, each of the pasteurized pear juices was aliquoted into separate sterile Duran bottles according to the following purposes: fermentation with *S. cerevisiae* or with *T. delbrueckii* or chemical analyses of the juices (three replicates for analyses).

The yeasts used in this study were propagated in sterile commercial YPD medium at 25 °C for 48 h under 150 rpm shaking. After 48 h of propagation, the colony populations of SC1116 and TD291 reached cell densities of 8.5×10^7 and 5.8×10^7 CFU/mL, respectively, as determined by the spread plate technique. Exact amounts of SC1116 (10.6 mL) or TD291 (16.6 mL) per 80 mL of pasteurized pear juice aliquots were calculated, collected, centrifuged, and washed with 0.9% sodium chloride solution (sterile) and then inoculated into the pasteurized pear juices at a yeast cell density of 10^7 CFU/mL. Fermentations were performed in duplicate at room temperature (total of 44 beverages; eleven pear cultivars × two yeast species × two fermentation replicates) in two replicates in this study. Fermentations were conducted in 500 mL Duran bottles using aliquots of 400 mL pasteurized pear juices. After fermentation, the beverages were centrifuged at 3000 × g for 10 min to remove any pear sediments and/or yeast cells. The obtained samples were aliquoted for sensory tests and replicate chemical analyses and kept at −20 °C (max. six months) until the analyses. The freezer storage was regarded to maintain the chemical compounds at approximately stable levels by preventing the breakdown of the chemical components and slowing down the metabolic processes (Mallik & Hamilton, 2017).

The fermentation kinetics were determined by the estimation of CO_2 production from bottle weight loss (every two days) during the fermentation process (Liu, Marsol-Vall, Laaksonen, Kortesniemi, & Yang, 2020). Stable °Brix values and no weight loss on two consecutive days were regarded as the completion of the fermentation process.

2.3. Measurements of pH, °Brix, sugars and organic acids, and major metabolites

The pH of the studied pear beverages was determined via a pH meter (WTW, Xylem Analytics Germany Sales GmbH & Co, Weilheim, Germany). The soluble solid concentrations (°Brix) were evaluated with a digital refractometer (Atago Co, Ltd., Tokyo, Japan).

The analysis of sugars and organic acids was conducted in duplicate based on the method of He et al. (2022b). An aliquot of pear sample (0.25 mL) was mixed with internal standards (myo-inositol for sugars and tartaric acid for organic acids, 0.25 mL of each) and diluted to a final volume of 5 mL with Milli-Q water. The diluted mixture was filtered with 0.45 μm PTFE filters, 0.3 mL of which was taken and evaporated under nitrogen flow (50 °C) until completely dry. After being kept in a P_2O_5 desiccator overnight, the dried sample was mixed with 0.6 mL of Tri-Sil reagent (Thermo Scientific, Pierce Biotechnology, Rockford, IL, USA), followed by 5 min of vortexing and 30 min of incubation at 60 °C. The sugars and organic acids in the studied beverage samples were analyzed as trimethylsilyl (TMS) derivatives by using a Shimadzu gas chromatograph (GC-2010Plus, Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector (FID) and a 0.25 μm SPB-1 column (30 m × 0.25 mm; Supelco, Bellefonte, PA, USA). The sugars and organic acids were identified by comparing the GC retention time with the external reference standards of sugars and organic acids. The concentrations of all standards were set to 5 g/L for quantification.

The major yeast metabolites (ethanol and glycerol) were determined in duplicate by a Shimadzu GC-FID equipped with a 0.25 μm HP-INNOWax column (30 m × 0.25 mm; Agilent, Santa Clara, CA, USA). The analysis conditions were the same as those in our previous study (He et al., 2021). The quantification of ethanol and glycerol was conducted by comparisons with authentic standards. The quantification of the analytes was carried out using external standard curves of different concentrations ($R^2 > 0.9999$).

2.4. Analysis of phenolic compounds

The phenolic compounds were extracted and analyzed using the method described in our previous study (He et al., 2022a). Approximately 25 mL of pasteurized pear juice and/or fermented pear beverage sample was extracted four consecutive times with 20 mL of ethyl acetate by sonication for 15 min. After centrifugation at 4500 × g for 15 min, the combined supernatants were evaporated to remove the ethyl acetate at 35 °C. The residues were dissolved in methanol (1.5 mL) and filtered through a 0.2 μm filter (PTFE).

The phenolic compounds in the pasteurized pear juices and fermented pear beverages were identified by a Bruker UHPLC-DAD-ESI-Q-TOF system (Bruker Corporation, Billerica, MA, USA), as previously described (He et al., 2022a). Briefly, the column was a Phenomenex Aeris peptide XB-C18 column (150 × 4.60 mm, 3.6 μm; Phenomenex, Torrance, CA, USA). A binary solvent system was used for the separation of the phenolic compounds in pasteurized pear juices and fermented pear beverages. Mobile phase A was 0.1% formic acid in Milli-Q water, and solvent B was 0.1% formic acid in acetonitrile. After DAD, 0.4 mL/min of LC eluent was directed to the ion source. Both positive and negative ion modes were used in the current study by applying the same method described in our previous study (He et al., 2022a). The mass was scanned from m/z 20 to m/z 2000 (full scan). The data were processed using Bruker Compass Data Analysis software version 4.4 (Bruker Corporation, Billerica, MA, USA). The pasteurized juice samples were analyzed in triplicate, and the fermented pear beverage samples were analyzed in duplicate for each fermentation by a Shimadzu UHPLC-DAD system (Shimadzu Corp., Kyoto, Japan). The analytical conditions (flow rate, chromatographic conditions, mobile phase solutions, and column) for the LC system were the same as those used in the identification analysis. The concentrations of the analytes were calculated by selected external standard curves.

2.5. Sensory evaluation of the studied fermented pear beverages

The sensory properties (flavor, taste, and appearance) of the fermented pear beverages were evaluated by thirteen panelists (10 women, 3 men, ages 21–56) with experience in sensory evaluation using a generic descriptive analysis. Prior to the tests, the panelists were informed about the procedures, samples, and treatments, and written consent was obtained. The panelists attended training sessions to develop a standard vocabulary to describe the fermented pear samples. A list of descriptors and reference standards (Supplementary Table S4) was agreed upon by the panel before the sensory evaluation. Twelve fermented pear beverages made from five experimental cultivars and one commercial pear cultivar (Table 1) and two yeasts were evaluated in triplicate in five sessions with 7 or 8 samples in each session presented in a randomized order (Williams Latin square design). The pear beverage samples (10 mL) were served in 50 mL transparent plastic beakers with glass lids at room temperature. The panelists were instructed to eat crackers and drink water to clean their palate after each sample. The intensities of each attribute were evaluated on line scales from 0 (none) to 10 (very strong) with the assistance of reference samples. The data were processed using Compusense Cloud software (version 8.4, Canada).

2.6. Statistical analysis

Pasteurized juice samples (n = 11 cultivars × 3 replicates = 33) and pear beverage samples (n = 11 cultivars × 2 yeasts × 2 replicate fermentations × 2 analysis replicates = 88) are represented in this study for the chemical data. Multivariate analysis was carried out using Unscrambler version 11 (Aspen Technology Inc., Bedford, MA). Multivariate models of principal component analysis (PCA) and partial least squares regression discrimination analysis (PLS-DA) were created to evaluate the differences among the pasteurized pear juices and pear beverages made with SC1116 and TD291. Two-way ANOVA models were applied to test the main effects and interactions of, first, fermentation treatment and cultivar and, second, yeast strain and cultivar on the main phenolic compound classes and sensory attributes. One-way ANOVA with Fisher's least significant difference (LSD) test was used to determine the significant differences in the concentrations of the 41 detected phenolic compounds among the cultivars and fermentations (main effects of the two-way ANOVA). Three-way ANOVA (12 samples, 13 panelists, 3 sessions, and their interactions) was applied for the results from the sensory panel with samples as a fixed factor and sessions and panelists as random factors. Statistical univariate analysis was conducted using IBM SPSS Statistics version 25.0 (SPSS Inc. H, Chicago, USA). All of the results are expressed as the means and standard deviations. The threshold of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Pear juice material types and fermentation kinetics

Fermentation kinetics were reflected by CO_2 production during yeast fermentations (carried out in duplicate), which was in accordance with our previous studies using other fruits (He et al., 2021; Liu et al., 2018). Due to the relatively low amounts of experimental pear juice material available for this study, the fermentations were carried out only in duplicate. The set of juices selected for this study is meant to represent pear materials in the early selection phase of a breeding program (sc., seedling field). In such highly diverse materials, a wide range of qualities is available, but the future cultivars are not spatially replicated in the field setup. Thus, more studies are needed in the future to fully characterize some of them as 'cultivars'. Rather, the cultivar differences observed in this study refer to potential fruit usage groups and can support breeders to categorize the breeding material for potential uses in novel types of juices and beverages. The kinetics of the two yeast species (SC1116 and TD291) varied significantly among the pear cultivars (Fig. 1). The cultivars 'Stolishnaja' ('Sto') and 'Conference' ('Con') had a faster fermentation rate, as indicated by their shorter fermentation times of 10 days (SC1116) and 14 days (TD291) when compared with the other cultivars. 'Sto' also showed lower amounts of CO_2 release during the fermentation processes (SC1116 at 6.8 g and TD291 at 8.1 g) than the other pear cultivars. This deviation might be ascribed to the lower sugar contents in the juice of 'Sto' relative to the other studied pear cultivars (He et al., 2022a). The yeast species also affected the fermentation process. SC1116 showed a faster fermentative performance than TD291 in most pear cultivars. All of the fermentation processes conducted using SC1116 were completed in or before 26 days, whereas those with TD291 took approximately 30 days. Both fermentations released similar amounts of CO_2, ranging from 6.8 to 15.8 g (SC1116) and 8.1 to 15.9 g (TD291).

3.2. Physicochemical characteristics of the pear beverages

The key physicochemical characteristics were determined from all fermented pear beverages (Table 2 and Table S1). The pH of the beverage samples ranged from 3.3 to 4.2, and they depended mainly on the cultivar differences. However, the impact of the yeast species on pH was not clear, since the difference between the average values of pH in the two types of pear beverages was not statistically significant. The total soluble solids (°Brix) were significantly affected by the yeast species, ranging from 3.2 to 7.1 in SC1116 pear beverages and 3.6–7.4 in TD291 pear beverages. The variation in °Brix degree was also associated with the pear cultivars. The ethanol contents ranged from 8.4% ('Py12') to 17.8% ('Py2') in beverage samples made with SC1116 and from 7.4% ('Py12') to 17.0% ('Py2') in TD291 beverage samples. In general, *S. cerevisiae*-fermented samples had higher ethanol contents (13.9% on average) than the *T. delbrueckii*-treated samples (12.8%). Interestingly, ethanol production did not directly comply with the production of CO_2 in the current study. A previous study demonstrated that the fermentation temperature may also alter yeast metabolism and divert CO_2 away from ethanol (Goold, Kroukamp, Williams, Paulsen, Varela, & Pretorius, 2017). For glycerol, fermentation using *T. delbrueckii* yeasts resulted in a significantly ($p < 0.05$) higher content (4.1 g/L on average) than fermentation using *S. cerevisiae* yeasts (3.0 g/L). This could have been due to the capacity of non-*Saccharomyces* yeasts to redirect sugar consumption from ethanol to other alternative compounds (glycerol and pyruvic acid) during alcoholic beverage fermentation (Ivit, Longo, & Kemp, 2020). In general, pear juices showed a lower glucose/fructose ratio and a higher sorbitol concentration than most apple juices (González Flores, Origone, Bajda, Rodríguez, & Lopes, 2021). A previous study investigated whether sorbitol facilitated yeast hexose transporter proteins, helping yeast strains to utilize the nonpreferred carbon sources (Jordan, Choe, Boles, & Oreb, 2016). Thus, the fermented pear beverages made from the original pear juices (without any carbon source addition) showed higher ethanol contents than those made from the original juices of apples or other fruits (He et al., 2021; Kelanne, Yang, Liljenbäck, & Laaksonen, 2020; Yang et al., 2021).

As shown in Table 2, the sugar and organic acid compositions were also highly dependent on the pear cultivars. The main residual sugar in the fermented pear beverages was sorbitol (ranging from 6.4 to 16.3 g/L in SC1116 samples and 7.2 to 21.4 g/L in TD291 samples), whereas other sugars were detected in small amounts. Sorbitol is regarded as the major sugar alcohol in the *Rosaceae* family (apples and pears), and it has been reported to contribute positively to sweetness perception in fruits and fruit products (Aprea et al., 2017; González Flores et al., 2021). In comparison to apple juices, pear juices have been reported to contain

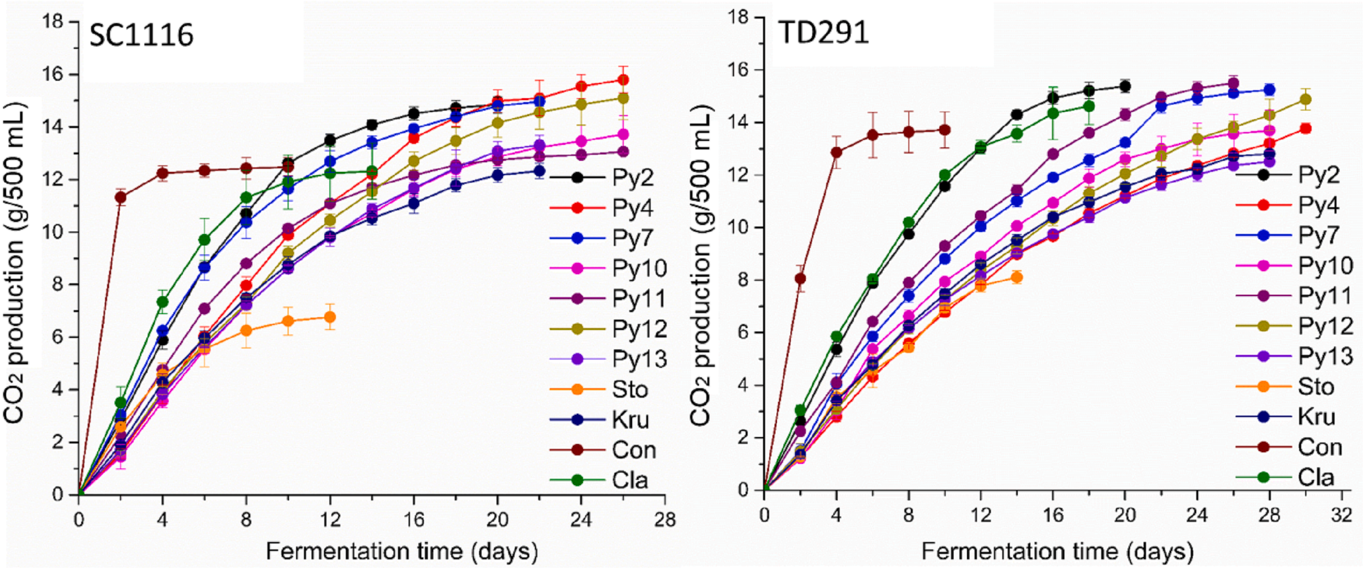

Fig. 1. Fermentation kinetics (expressed as CO_2 emissions) of *S. cerevisiae* 1116 and *T. delbrueckii* 291 in pear beverages. The stable °Brix values and no CO_2 release on two consecutive days indicated the completion of fermentation process.

Table 2
Major compositional characteristics of fermented pear beverages made from SC1116 and TD291.

Cultivars	Sorbitol (g/L)	Sum of sugars (g/L)	Succinic acid (g/L)	Malic acid (g/L)	Sum of organic acids (g/L)	pH values	°Brix	Ethanol (%)	Glycerol (g/L)
SC1116 pear beverages									
Py2	9.0 ± 0.1b	9.2 ± 0.0b	1.5 ± 0.1b	5.7 ± 0.2f	8.9 ± 0.4e	3.6 ± 0.0c	5.5 ± 0.1c	17.8 ± 0.2f	3.5 ± 0.1b
Py4	13.4 ± 0.1d	14.0 ± 0.1d	1.3 ± 0.1b	3.2 ± 0.1c	6.8 ± 0.2 cd	3.6 ± 0.0c	5.5 ± 0.0c	16.4 ± 0.1e	3.5 ± 0.4b
Py7	12.5 ± 0.3d	12.9 ± 0.2d	1.7 ± 0.0c	4.2 ± 0.1d	7.0 ± 0.3d	3.4 ± 0.0b	4.9 ± 0.1b	16.3 ± 0.2e	3.5 ± 0.2b
Py10	11.1 ± 0.1 cd	11.6 ± 0.1c	1.1 ± 0.1a	4.8 ± 0.1e	7.1 ± 0.1d	3.7 ± 0.0d	6.1 ± 0.1d	12.4 ± 0.2b	2.7 ± 0.1ab
Py11	15.8 ± 0.1e	16.5 ± 0.1e	1.8 ± 0.1d	2.8 ± 0.1bc	6.3 ± 0.2c	3.6 ± 0.0d	5.6 ± 0.0c	12.5 ± 0.1b	2.8 ± 0.1ab
Py12	16.3 ± 0.1e	16.7 ± 0.1e	2.0 ± 0.1e	2.3 ± 0.0b	6.3 ± 0.1c	3.6 ± 0.0d	7.1 ± 0.0e	8.4 ± 0.1a	2.3 ± 0.2a
Py13	10.4 ± 0.3c	11.3 ± 0.3c	1.1 ± 0.2a	2.2 ± 0.1b	4.6 ± 0.2a	3.7 ± 0.0d	4.8 ± 0.0b	14.6 ± 0.2d	2.7 ± 0.3ab
Sto	6.6 ± 0.5a	7.1 ± 0.5a	1.0 ± 0.1a	3.8 ± 0.1d	6.8 ± 0.2 cd	3.3 ± 0.0a	3.2 ± 0.0a	12.6 ± 0.0b	2.4 ± 0.1a
Kru	10.5 ± 0.1c	11.2 ± 0.2c	1.0 ± 0.1a	1.6 ± 0.1a	4.4 ± 0.6a	3.5 ± 0.0c	4.5 ± 0.0b	13.2 ± 0.1c	3.7 ± 0.1b
Cla	16.4 ± 1.4e	16.7 ± 1.4e	1.5 ± 0.1b	2.7 ± 0.2bc	5.6 ± 0.3b	4.1 ± 0.0f	5.7 ± 0.0c	14.6 ± 0.6d	2.8 ± 0.3ab
Con	9.0 ± 0.3b	9.5 ± 0.3b	1.4 ± 0.1b	2.4 ± 0.1b	4.9 ± 0.2a	3.9 ± 0.0e	5.8 ± 0.4 cd	14.2 ± 0.1d	2.9 ± 0.1ab
aver	11.9A	12.4A	1.4A	3.3A	6.3A	3.6A	5.3A	13.9B	3.0A
TD291 pear beverages									
Py2	8.8 ± 0.1b	9.2 ± 0.1b	2.2 ± 0.0c	5.2 ± 0.1e	9.1 ± 0.1e	3.6 ± 0.0c	5.8 ± 0.0d	17.0 ± 0.1e	5.6 ± 0.1e
Py4	13.0 ± 0.1d	13.9 ± 0.1c	1.9 ± 0.1b	3.5 ± 0.1d	6.5 ± 0.1bc	3.6 ± 0.0c	5.5 ± 0.0c	15.8 ± 0.1d	4.1 ± 0.1b
Py7	12.7 ± 0.1d	13.3 ± 0.1c	2.4 ± 0.1c	4.1 ± 0.1d	7.7 ± 0.2d	3.5 ± 0.0b	5.1 ± 0.0b	15.4 ± 0.1d	4.2 ± 0.1b
Py10	12.1 ± 0.2d	12.9 ± 0.2c	1.4 ± 0.1a	5.1 ± 0.1e	7.8 ± 0.1d	3.6 ± 0.0c	6.2 ± 0.0d	11.4 ± 0.1b	4.0 ± 0.0b
Py11	17.0 ± 0.1e	18.1 ± 0.1d	2.3 ± 0.1c	3.0 ± 0.1c	7.0 ± 0.1c	3.6 ± 0.0c	7.1 ± 0.0e	11.5 ± 0.1b	3.7 ± 0.6b
Py12	18.1 ± 0.1e	18.8 ± 0.1d	2.8 ± 0.2d	2.4 ± 0.1bc	7.2 ± 0.2c	3.6 ± 0.0c	7.4 ± 0.0f	7.4 ± 0.2a	2.9 ± 0.1a
Py13	12.2 ± 0.2d	13.5 ± 0.2c	1.5 ± 0.1a	2.1 ± 0.0b	4.7 ± 0.1a	3.8 ± 0.0d	4.9 ± 0.0b	13.0 ± 0.0c	3.8 ± 0.1b
Sto	7.2 ± 0.5a	7.6 ± 0.5a	1.5 ± 0.2a	3.9 ± 0.4d	7.3 ± 0.7 cd	3.3 ± 0.0a	3.6 ± 0.0a	12.2 ± 0.1b	2.6 ± 0.1a
Kru	10.7 ± 0.3c	11.8 ± 0.2c	1.8 ± 0.2ab	1.4 ± 0.2a	4.5 ± 0.0a	3.7 ± 0.0c	4.6 ± 0.1b	12.1 ± 0.1b	5.1 ± 0.1d
Cla	21.4 ± 1.3f	21.6 ± 1.2d	1.7 ± 0.1ab	2.7 ± 0.0c	5.6 ± 0.2b	4.2 ± 0.0e	5.9 ± 0.1d	11.9 ± 0.2b	5.0 ± 0.3c
Con	12.5 ± 1.1d	12.9 ± 1.1c	1.8 ± 0.1b	2.6 ± 0.1c	5.7 ± 0.6b	3.9 ± 0.0d	7.0 ± 0.0e	13.3 ± 0.1c	4.4 ± 0.1bc
aver	13.2B	13.9B	1.9B	3.3A	6.6A	3.3A	5.8B	12.8A	4.1B

Results are presented as the means (duplicate measurements of duplicate fermentations). ND: not found.
For pear beverages produced with same yeast, significant differences among those beverages are presented in lower case letters a-h; for average contents of pear beverages made with different yeasts, significant differences are presented in upper case letters A-B ($p < 0.05$, One-way ANOVA with Fisher's least significant difference test).
Abbreviations of pear cultivar names refer to Table 1. Sum of sugars was calculated by the sum of fructose, glucose, sucrose, sorbitol, and xylose sum of organic acids was calculated by the sum of quinic acid, succinic acid, malic acid, citric acid, and ascorbic acid Numeric indicators of chemical compounds were: sorbitol (46), succinic acid (48), malic acid (49), ethanol (53), and glycerol (54).

higher concentrations of sorbitol (González Flores et al., 2021). The high concentrations of sorbitol in the studied pear beverages can be derived from the corresponding original pear juices, as previously reported (González Flores et al., 2021). In general, the pear beverages made with TD291 (14.0 g/L on average) contained significantly higher concentrations ($p < 0.05$) of residual sugars than those made with SC1116 (12.4 g/L on average). The lower sugar consumption capacity and lower conversion of sugars to ethanol by *T. delbrueckii* was consistent with a previous study with bilberry beverages (Liu et al., 2018). Sorbitol is usually incompletely absorbed by the human body, unlike 'regular sugars', such as sucrose and glucose (Jungo, Schenk, Pasquier, Marison, & von Stockar, 2007). Thus, it is a popular sweetener used for low-carbohydrate diets. However, the overconsumption of sorbitol could result in bloating, diarrhea, and flatulence, as it is not absorbed in the small intestine. Thus, it is important to acknowledge the contents when selecting cultivars for beverage production and develop yeasts to reduce sorbitol levels in the obtained pear beverages.

Malic acid was detected as the main residual organic acid in the pear beverages, followed by succinic, quinic, ascorbic, and citric acids. The malic acid contents ranged from 1.6 g/L ('Kru') to 5.7 g/L ('Py2') in the SC1116 samples and from 1.4 g/L ('Kru') to 5.2 g/L ('Py2') in the TD291 samples. As the major yeast-derived organic acid, succinic acid was mainly influenced by the yeasts. It showed significantly higher concentrations ($p < 0.05$) in the TD291 samples (1.9 g/L on average) than in the SC1116 samples (1.4 g/L on average). These results indicated that the total organic acid contents were mainly dependent on the pear cultivars, whereas the yeasts only significantly affected the succinic acid content of the five analyzed organic acids.

3.3. Analysis of the phenolic compounds

Altogether, 41 phenolic compounds were identified from the juices and fermented pear beverages by UHPLC-DAD-ESI-QTOF, including 19 hydroxycinnamic acids, 3 hydroxybenzoic acids, 11 flavonols, 2 flavan-3-ols, 5 procyanidins, and arbutin. Phenolic acids in the pear samples mainly presented as derivatives of coumaric, caffeic, ferulic, syringic, and sinapic acids, whereas caffeic, coumaric, and syringic acids were also found in their free forms (Supplementary Table S2 and Fig. S1). Coumaric acid (Compound 16) was detected only in the fermented samples, which might be due to the hydrolysis of coumaric acid derivatives during fermentation. The flavan-3-ols detected in the samples were (+)-catechin and (-)-epicatechin. Flavonols were identified mainly as glycosides of quercetin, isorhamnetin, and kaempferol, whereas quercetin (Compound 42) was found only in the fermented samples. Moreover, both A- and B-type procyanidins were identified in the pear juices and beverages.

In general, the pasteurization process altered the phenolic compositions and profiles, which was significantly dependent on the different pear cultivars. The sum of the quantified phenolic contents is shown in Table 3. In comparison to the untreated pear juices (He et al., 2022a), the pasteurization process led to a decrease (21%) in the sum of quantified phenolic contents. As phenolic compounds are heat-sensitive compounds, they are unstable and easily degradable under thermal treatments (Rawson et al., 2011). A similar reduction in phenolic compounds has been previously reported for apple and orange juices (Pala & Toklucu, 2013). As previously reported, the sum of quantified phenolic contents is closely related to α-glucosidase inhibitory (α-GI) activities (Adyanthaya, Kwon, Apostolidis, & Shetty, 2010). In other words, high α-GI activities correlated with a high sum of quantified phenolic contents in the juices. The α-GI activities might be relatively high in the untreated juices made from pear breeding selections (He et al., 2022a), whereas the pasteurization process used in the current study might have led to a decrease in the α-GI activities of the fruit juices (Alongi et al., 2019). Moreover, this result could also have resulted from the thermal inactivation of polyphenol oxidase (PPO), preventing the phenolic compounds from degradation by those browning reactions (Alongi, Verardo, Gorassini, & Anese, 2018). Overall, the reactions during the pasteurization process are complex and require more investigation. In addition, the effects of pasteurization varied among the different pear cultivars, which might indicate differences in the α-GI or PPO activities in the different pear cultivars.

The fermentation process led to a decrease in the sum of identified hydroxybenzoic acids (by 4.1%), procyanidins (by 14.9%), and flavonols (by 9.6%), whereas the sum of flavan-3-ols (by 33.5%) and hydroxycinnamic acids (by 30.9%) was increased. In general, the pear beverages (fermented by both yeasts) contained significantly ($p < 0.05$) higher concentrations of quantified hydroxycinnamic acids than the corresponding juices. As shown in Table 2, the significant differences between the fermented pear beverages and juices were mainly in the average concentrations of caffeic acid derivatives and coumaric acid derivatives, primarily 5-*O*-caffeoylquinic acid, di-*O*-caffeoylquinic acid isomer I, di-*O*-caffeoylquinic acid isomer II, caffeoylshikimic acid, and coumaroylquinic acid isomers I & II, together with free caffeic and coumaric acids. Similar results were also found in Kei-apple beverages produced from *S. cerevisiae*, and the concentrations of the detected phenolic acids in the Kei-apple beverages were 25% higher than those in the corresponding juices (Minnaar, Jolly, Paulsen, Du Plessis, & Van Der Rijst, 2017). The increase in the sum of quantified phenolic contents in pear beverages can be ascribed to the release of bound phenolic compounds from polysaccharides and mannoproteins by the yeast species. Fermentation leads to hydrolysis reactions of the esterified forms of free hydroxycinnamic acids and various polysaccharides and mannoproteins (Kulkarni et al., 2015). The increase in the concentrations of detected flavan-3-ols and the decrease in the detected amounts of procyanidins could be due to the hydrolysis of procyanidins by yeasts during alcoholic pear beverage fermentation (Vidal, Cartalade, Souquet, Fulcrand, & Cheynier, 2002). Similarly, the hydrolysis of glycosylated flavonol compounds decreases the concentrations of detected flavonol glycosides and increases the contents of quercetin aglycone in grape wine (Makris, Kallithraka, & Kefalas, 2006).

In general, the phenolic compositions and profiles of the fermented pear beverages depended mainly on the pear cultivars (Table 3, Supplementary Table S3). The sum of quantified phenolic contents of SC1116 pear beverages ranged from 'Cla' (142 mg/L) to 'Py2' (649 mg/L), whereas that of TD291 pear beverages ranged from 'Cla' (144 mg/L) to 'Py2' (698 mg/L). Interestingly, the pasteurized juices made from 'Py10' contained the highest phenolic contents (614 mg/L) detected in this study. Although they still contained a relatively high sum of quantified phenolic contents in the corresponding pear beverages, the increase in the sum of quantified phenolic contents in the pear beverages produced from either of the two yeasts was not significant ($p > 0.05$) when compared with the corresponding pasteurized pear juices. In addition, the differences between the pasteurized pear juices and their pear beverage products made from pear cultivars and breeding selections 'Py13', 'Sto', and 'Cla' were not significant. The yeast species also played an important role in the phenolic compositions and profiles of the final pear beverage products. In general, fermentation with TD291 showed a slightly higher ($p < 0.05$) retaining capacity of hydroxycinnamic acids, such as 5-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid, as well as quercetin-3-*O*-glucoside, when compared with SC1116.

To visualize the differences between the pear beverages and their corresponding pasteurized juices based on their phenolic compound contents, partial least squares discriminant analysis (PLS-DA) was carried out using samples (Y-data, $n = 3$, for pear juices and two fermented pear beverages) and phenolic compound variables (X-data, $n = 47$). In the PLS-DA plots of Fig. 2A, 54% of the chemical variables explained 42% of the variation in the three factors ($R^2 = 93.86\%$, validated $R^2 = 91.58\%$). The validated correlation coefficient number was quite high in this model, which can be due to the fewer factors associated with the separation of pear juices and fermented pear beverages. Factor-1 clearly separated the pasteurized pear juices from the fermented pear beverages. The pear juices were negatively correlated with coumaric acid (16), coumaroylquinic acid isomer I (20), and total concentrations of flavan-3-ols due to the low contents of the compounds detected in these juice samples. However, this model was not able to demonstrate differences in the phenolic profiles between pear beverages produced by different yeasts (SC1116 and TD291).

The overall effects of the phenolic compositions and the studied pear products were further investigated using PCA models (Fig. 2B & 2C). As shown in the PCA model (Fig. 2B), pear breeding selections 'Py2' and 'Py10' were clearly separated from the others along PC-1, showing a strong correlation with arbutin (Compound 1); flavan-3-ols; procyanidins, primarily A-type procyanidin dimer (2), B-type procyanidin dimer (4), (+)-catechin (5), and procyanidin dimer B2 (6); hydroxycinnamic acids, mainly caffeoyl *N*-tryptophan (13), 5-*O*-caffeoylquinic acid (15), 4-*O*-caffeoylquinic acid (18), caffeoylshikimic acid (22), coumaroylquinic acid isomer II (23), feruloylquinic acid isomer I (26), and ferulic acid derivative (29); sinapic acid hexoside II (30); and flavonols, such as quercetin hexoside (34), quercetin-3-*O*-glucoside (35), isorhamnetin hexoside II (39), and isorhamnetin-acylated-hexoside II (41). In addition, the pear cultivar 'Sto' was positively correlated with hydroxybenzoic acids and flavonols on PC-2, such as syringic acid (8), syringic acid hexoside II (9), quercetin hexoside deoxyhexoside II (33), and isorhamnetin hexoside deoxyhexoside (36). Similar results were also found in the untreated pear juices made from these three cultivars ('Py2', 'Py10', and 'Sto'), as previously reported in our pear juice study (He et al., 2022a). Dessert pears are typically popular due to their good flavors, whereas astringent pears are suitable for making 'perry' products rich in bioactive compounds such as polyphenols. In general, pasteurized pear juices and fermented pear beverage products made from cultivars of the 'perry usage group' ('Py10' and 'Sto') were

Table 3

Summarization of major phenolic compound concentrations (mg/L) in pasteurized pear juice and fermented pear beverage samples produced with *S. cerevisiae* 1116 and *T. delbrueckii* 291.

Pears	Sum of hydroxybenzoic acids	Sum of flavan-3-ols	Sum of proanthocyanins	Sum of flavonols	Sum of hydroxycinnamic acids	Arbutin	Sum of phenolic compounds
Pasteurized pear juices							
Py2	28.7 ± 2.9b	35.5 ± 4.5de	76.4 ± 5.0f	90.5 ± 3.7 g	299.5 ± 8.4e	31.8 ± 1.6c	562.4 ± 17.6 g
Py4	30.7 ± 1.4b	59.4 ± 2.1f	58.3 ± 7.2d	42.2 ± 2.3e	220.3 ± 14.6d	9.7 ± 0.1b	390.7 ± 19.4f
Py7	22.3 ± 2.6a	31.7 ± 3.5d	47.3 ± 4.5c	45.7 ± 5.3e	187.4 ± 10.3c	11.5 ± 0.5b	346.1 ± 15.5e
Py10	33.4 ± 1.2b	53.5 ± 7.9f	115.4 ± 3.9 g	91.1 ± 4.4 g	310.0 ± 18.1e	10.9 ± 0.1b	614.2 ± 29.8 h
Py11	40.3 ± 3.1c	57.9 ± 6.0f	64.6 ± 3.4e	26.0 ± 1.1c	90.0 ± 5.2d	6.4 ± 0.1a	285.2 ± 10.5d
Py12	29.9 ± 3.6b	29.0 ± 2.6d	54.9 ± 3.1d	8.7 ± 1.1a	87.5 ± 5.0d	4.5 ± 0.2a	214.5 ± 10.5c
Py13	20.8 ± 3.3a	17.9 ± 2.0b	29.8 ± 2.5ab	34.0 ± 2.2d	39.0 ± 1.4a	10.4 ± 0.1b	151.9 ± 7.3ab
Sto	69.7 ± 6.2c	37.5 ± 0.7e	29.0 ± 3.6ab	62.4 ± 4.4f	199.4 ± 5.0c	9.1 ± 0.3b	407.0 ± 10.8f
Kru	17.1 ± 3.2a	10.5 ± 1.4a	23.3 ± 2.6a	35.9 ± 2.4d	47.7 ± 4.6b	4.1 ± 0.4a	138.7 ± 12.8a
Cla	27.4 ± 0.4b	22.5 ± 1.9c	27.9 ± 3.1ab	17.2 ± 1.5b	28.7 ± 1.1a	6.1 ± 0.1a	129.8 ± 5.1a
Con	21.6 ± 2.9a	14.8 ± 1.3ab	34.8 ± 4.7b	50.1 ± 2.2e	36.0 ± 6.9a	6.9 ± 0.5a	164.2 ± 8.8b
aver	31.1A	30.9A	51.1B	45.8B	140.5A	10.1A	309.5A
SC1116 pear beverages							
Py2	27.8 ± 2.1b	47.0 ± 5.4c	71.7 ± 5.2e	82.9 ± 5.2 h	383.7 ± 9.6i	36.6 ± 1.0d	649.6 ± 14.6 g
Py4	26.3 ± 1.0b	36.5 ± 2.9b	48.1 ± 1.3 cd	39.6 ± 1.1e	296.5 ± 9.1 g	10.7 ± 0.3c	457.7 ± 9.0f
Py7	24.6 ± 3.0ab	43.4 ± 5.3c	40.1 ± 2.6c	41.6 ± 1.4e	243.0 ± 13.5f	10.6 ± 0.4c	403.3 ± 14.7e
Py10	34.3 ± 2.3c	62.2 ± 5.8d	100.7 ± 2.6f	74.8 ± 3.5 g	341.3 ± 16.2 h	9.9 ± 0.4c	623.2 ± 17.4 g
Py11	37.4 ± 4.8c	71.7 ± 3.6e	52.1 ± 2.5d	22.8 ± 1.6c	137.2 ± 8.8d	7.0 ± 0.3b	328.3 ± 13.6d
Py12	27.7 ± 3.0b	38.8 ± 2.6b	46.9 ± 5.4 cd	7.7 ± 1.0a	132.7 ± 6.8d	4.9 ± 0.1a	258.7 ± 10.5c
Py13	22.5 ± 1.9a	26.1 ± 3.8a	21.0 ± 1.4a	29.6 ± 1.4d	61.4 ± 4.7b	8.5 ± 0.3bc	169.0 ± 10.7b
Sto	64.8 ± 5.5d	52.0 ± 4.5c	25.4 ± 3.5ab	56.0 ± 3.4f	205.0 ± 10.6e	7.9 ± 0.4bc	411.0 ± 8.1e
Kru	17.7 ± 2.6a	20.2 ± 2.3a	19.4 ± 1.5a	31.7 ± 3.5d	77.2 ± 2.7c	5.3 ± 0.3a	171.3 ± 5.9b
Cla	26.9 ± 2.4b	34.5 ± 3.2b	22.9 ± 1.8a	15.0 ± 1.1b	36.6 ± 2.4a	6.2 ± 0.2b	142.0 ± 5.9a
Con	21.6 ± 2.3a	24.1 ± 1.2a	30.9 ± 0.6b	42.1 ± 1.4e	61.1 ± 8.1b	6.9 ± 0.5b	186.7 ± 8.5b
aver	30.1A	41.3B	43.6A	40.5A	179.2B	10.4A	345.1B
TD291 pear beverages							
Py2	28.9 ± 1.2b	46.4 ± 5.7c	71.4 ± 3.3f	87.0 ± 4.6 g	429.0 ± 6.3i	35.0 ± 0.6e	697.7 ± 14.8 h
Py4	28.7 ± 1.9b	37.0 ± 2.4b	49.8 ± 2.9de	39.3 ± 3.6e	309.2 ± 15.7 g	4.3 ± 0.2a	468.4 ± 15.0f
Py7	23.0 ± 1.4a	41.9 ± 5.7bc	38.5 ± 2.4c	42.4 ± 2.2e	247.4 ± 8.1f	11.1 ± 0.7d	404.5 ± 15.5e
Py10	32.1 ± 2.5bc	62.1 ± 2.4d	97.9 ± 5.7f	79.5 ± 8.7f	346.3 ± 15.4 h	10.7 ± 0.3d	628.5 ± 16.4 g
Py11	36.0 ± 2.8c	68.3 ± 2.0d	55.6 ± 4.6e	24.5 ± 1.4c	137.7 ± 6.1d	6.04 ± 0.47b	328.2 ± 12.4d
Py12	28.1 ± 2.4b	38.4 ± 3.7b	45.6 ± 0.6d	8.4 ± 0.1a	135.2 ± 7.5d	5.3 ± 0.1a	260.9 ± 12.8c
Py13	19.8 ± 1.6a	25.2 ± 0.5a	21.9 ± 2.3a	31.1 ± 1.5d	70.4 ± 6.2bc	8.6 ± 0.4c	177.1 ± 14.5b
Sto	61.8 ± 4.1d	48.8 ± 2.8c	26.8 ± 2.3b	61.0 ± 4.4f	218.2 ± 7.9e	8.0 ± 0.2c	424.7 ± 10.4e
Kru	18.6 ± 2.3a	23.6 ± 0.4a	18.0 ± 2.2a	33.4 ± 1.6d	80.2 ± 3.9c	5.1 ± 0.2a	179.0 ± 4.5b
Cla	27.3 ± 2.4b	36.0 ± 0.4b	22.3 ± 2.2a	15.1 ± 1.1b	36.9 ± 3.1a	6.4 ± 0.6b	144.0 ± 10.4a
Con	20.6 ± 2.3a	23.8 ± 2.1a	29.1 ± 3.2b	45.6 ± 1.4e	60.2 ± 5.9b	6.6 ± 0.3b	185.9 ± 13.5b
aver	29.6A	41.1B	43.4A	42.4A	188.2B	9.8A	354.4B
Two-way ANOVA model 1[a]							
Fermentation	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fermentation × cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Two-way ANOVA model 2[a]							
Yeast	<0.001	0.026	ns	<0.001	<0.001	<0.001	<0.001
Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Yeast × cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Results are presented as the means (triplicate samples for juices; four pseudoreplicates for fermented pear beverage samples). ND: not found. For pasteurized juices or beverages produced with same yeast, significant differences among cultivars within product group are presented in lower case letters a-h ($p < 0.05$); for average contents per compound group amongst juice and pear beverages made with different yeasts, significant differences are presented in upper case letters A-B ($p < 0.05$; One-way ANOVA with Fisher's least significant difference test).

Abbreviations of pear cultivar names refer to Table 1, category of each phenolic group is shown in Table S2.

[a] Two-way ANOVA, first model: fermentation = juice samples *vs* fermented beverage samples (two classes), cultivar (11); second model: yeast = SC1116 samples *vs* TD291 samples (two classes), cultivar (11).

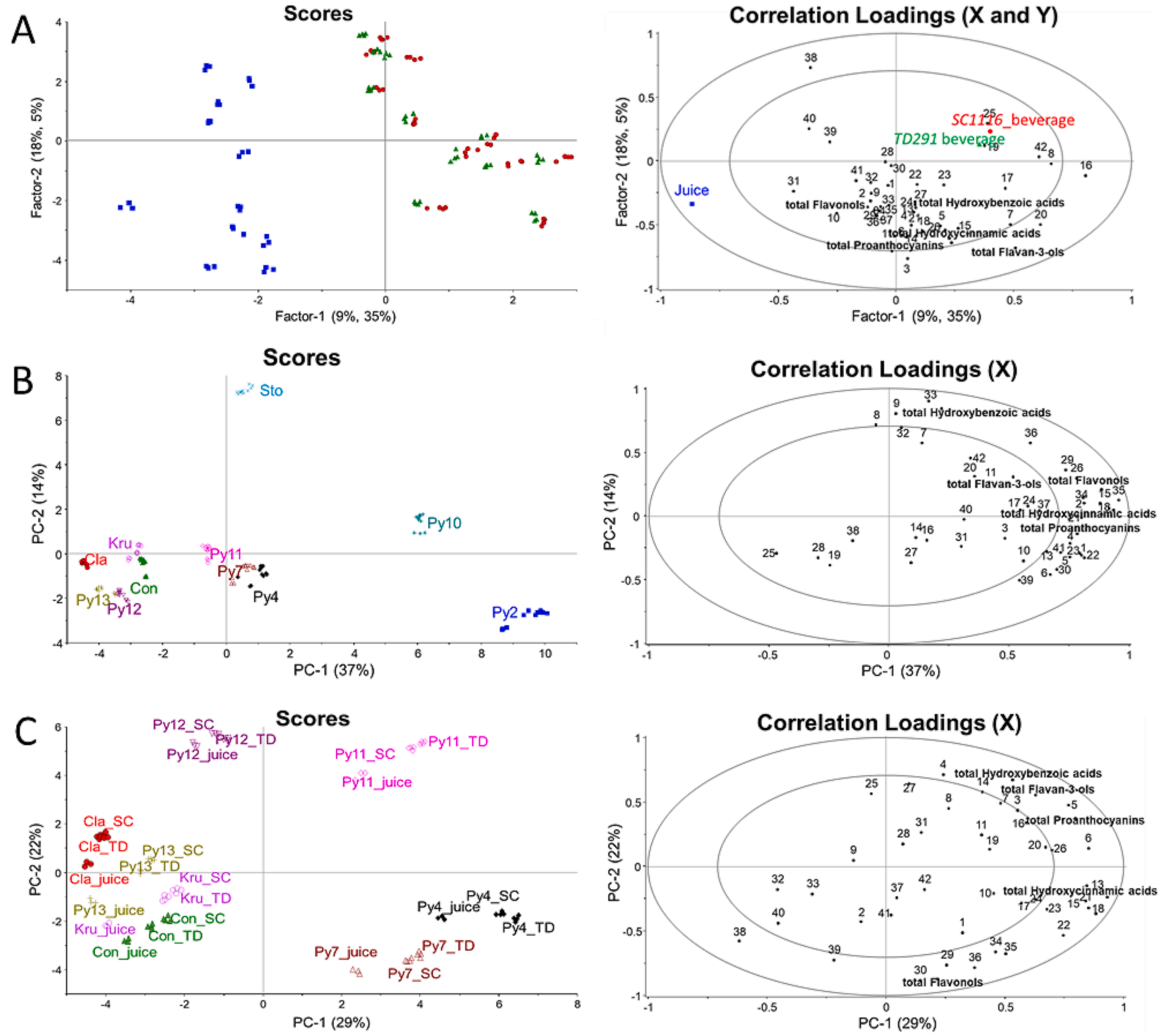

Fig. 2. Multivariate models of the phenolic composition of the pasteurized pear juices and fermented pear beverages. A: PLS-DA figure with all cultivars; B: PCA figure with all cultivars; C: PCA figure where dominant cultivars 'Py2', 'Py10', and 'Sto' were excluded. In Fig. 2A, pasteurized juices with rectangles (blue), SC1116 pear beverages with triangles (green), and TD291 pear beverages with circles (red). In Fig. 2B and 2C, pear cultivars are presented with different symbols and colors. The abbreviations of pear cultivar names are shown in Table 1, and the numeric indicators of phenolic compounds are shown in Supplementary Table S4.

correlated with higher concentrations of phenolic compounds.

The second PCA model was established by removing the three dominant cultivars ('Py10', 'Py12′, and 'Sto') to show the differences among the rest of the other pear cultivars. As shown in Fig. 2C, this PCA model resulted in 51% of the variance explained by PC1 (29%) and PC2 (22%). In this PCA model, pear cultivars and breeding selections of the dessert types 'Py13′, 'Cla', 'Con', and 'Kru' were grouped together and located on the left side of PC-1, showing strong correlations with isorhamnetin hexoside I (38). Pear breeding selections 'Py4′ and 'Py7′ were located on the negative side of PC-2, showing strong correlations with caffeoyl *N*-tryptophan (Compound 13, Supplementary Table S2), 5-*O*-caffeoylquinic acid (15), 4-*O*-caffeoylquinic acid (18), caffeoylshikimic acid (22), coumaroylquinic acid isomer II (23), ferulic acid derivative (29), and sinapic acid hexoside II (30), as well as flavonols, mainly quercetin hexoside (34), quercetin-3-*O*-glucoside (35), and isorhamnetin hexoside deoxyhexoside (36). In comparison to Fig. 2B, the PCA models clearly separated the pear cultivars 'Py7', 'Py11', 'Py12', and 'Py4' from the other pear cultivars, which were determined to be 'dessert usage pears' ('Py13', 'Kru', 'Con', and 'Cla'). The fruits of 'Py7' and 'Py11' were evaluated beforehand by breeders as cultivars of the 'perry/dessert usage group'; indeed, their juices contained higher concentrations of phenolic compounds than the 'dessert usage pears'. In addition, 'Py12' was determined to be a cultivar in the 'perry usage group', and 'Py4' was determined to be a cultivar in the 'dessert usage group'. This result is in accordance with our previous study on the chemical compositions of these pear juice samples (He et al., 2022a).

3.4. *Sensory profiles of the fermented pear beverages*

The sensory properties of the selected pear beverages produced from six pear cultivars and breeding selections ('Py4', 'Py10', 'Py12', 'Sto', 'Kru', and 'Cla') were studied with a trained sensory panel. Samples were selected based on preliminary chemical analyses (data not shown), representing large variation within samples. In the three-way ANOVA, significant sample main effects were observed for ‘pear’ and ‘cooked pear’ odors and for the taste attributes (sweetness, bitterness, sourness) and astringency. In general, the sensory profiles of the studied pear beverages were highly dependent on the pear cultivars, whereas the yeast had notably fewer effects (Supplementary Table S6). For ‘cooked pear’ and ‘floral’ odors and sweet taste, the samples made using TD291 were evaluated as more intense than the SC1116 samples. The effect of the pear cultivar can be observed in the sensory consensus plot PCA in Fig. 3A, where samples made from ‘Sto’ (by either yeast strain) were located on the right side of PC-1 together with the astringency, sourness, and ‘pear’ odor variables. The astringency and sourness in the fermented pear beverages made from ‘Sto’ might be ascribed to the fruit characteristics described as acidic and astringent by breeders. Pear beverages made from ‘Py10’ (again by both yeasts) correlated highly with ‘pear’ and ‘acidity’ odors and a sour taste. Moreover, pear beverages made from the commercial cultivar ‘Cla’ were highly related to its sweet taste. Generally, alcoholic pear beverages with higher astringency and sourness were made from sour and astringent pears, whereas sweet pear beverages were made from sweet and dessert pear cultivars. The appearances of the pear beverages also showed high correlations with the pear cultivars. The PCA model (where 60% of the variables were included in PC-1 and PC-2) in Fig. 3B shows that the pear beverages produced from ‘Sto’ and ‘Kru’ were characterized as clear, brown, and pale, whereas those made from ‘Py4’, ‘Py10’, ‘Py12’, and ‘Cla’ were characterized as white, yellow, and cloudy.

The relationships between the sensory profiles and phenolic compounds were further investigated via multivariate models in the current study. As shown in the PCA model (Fig. 3C), higher concentrations of hydroxybenzoic acids (Compounds 8 and 9, Supplementary Table S2), monomeric and polymeric flavan-3-ols (2 and 7), hydroxycinnamic acids (20 and 26), sinapic acid hexoside I (21), and feruloylquinic acid isomer I (26), as well as the main flavonol glycosides (32–34, 36) and free quercetin (42), correlated closely with astringency perception. The contribution of hydroxycinnamic acids and procyanidins to astringency perception has also been demonstrated well in our apple cider study (He et al., 2022b). In addition, the sweetness perception of pear products in

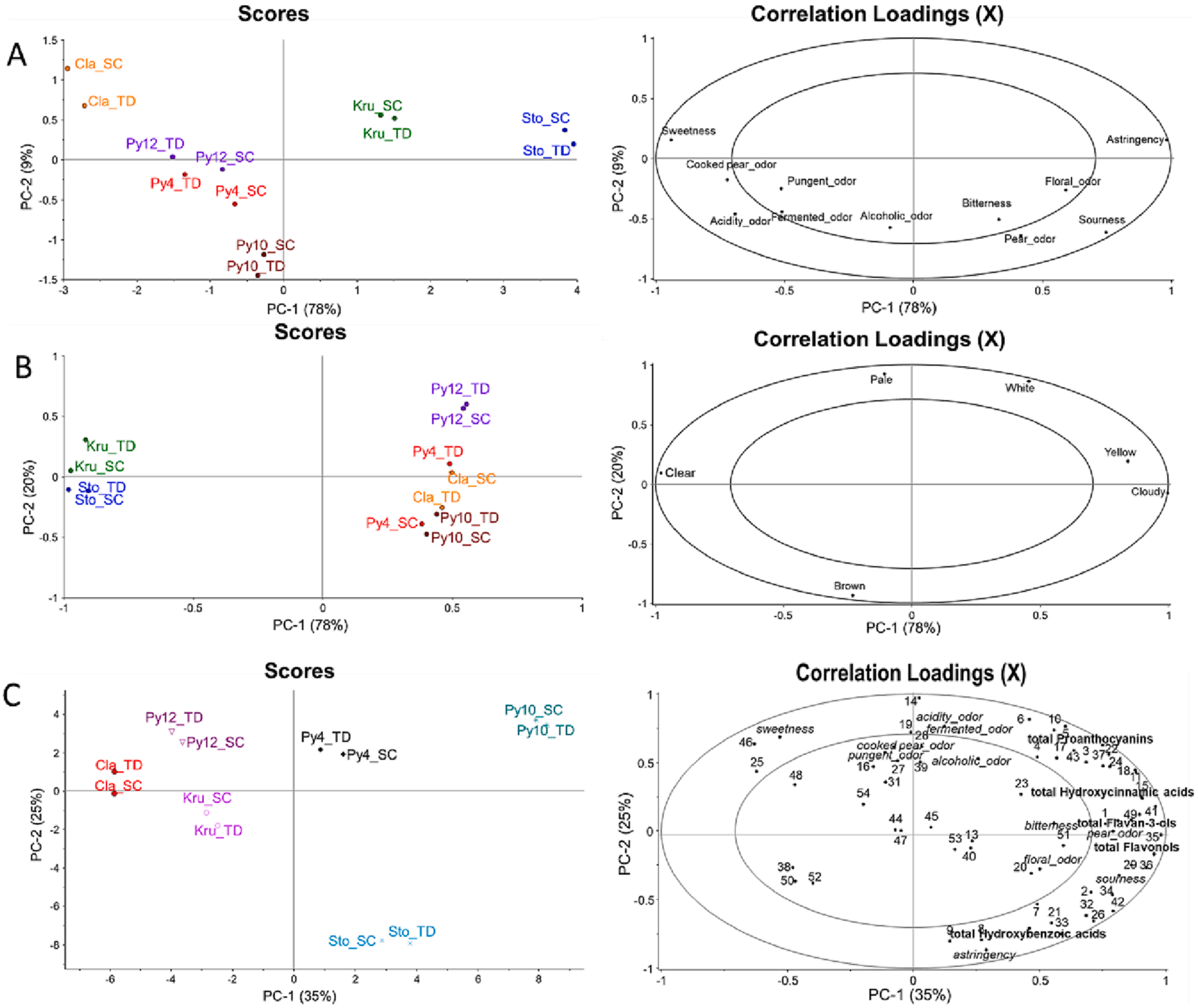

Fig. 3. PCA models of sensory attributes and their correlations with phenolic compositions in selected pear beverages (‘Py4’, ‘Py10’, ‘Py12’, ‘Sto’, ‘Kru’, and ‘Cla’ produced from SC1116 and TD291). A: Rated pleasantness and flavor and odor attributes; B: CATA attributes for appearance; C: Correlations between chemical compositions and sensory properties (flavor and odor attributes). Pear cultivars are presented with different symbols and colors. The abbreviations of pear cultivar names are shown in Table 1, and the numeric indicators of phenolic compounds are shown in Supplementary Table S4.

the current study was highly correlated with di-*O*-caffeoylquinic acid I (Compound 25) and sorbitol (46). Residual sorbitol was found to be the main contributor to the sweetness of the fermented pear beverage products. No significant relationship was found between the phytochemical compounds and bitterness in the current study.

4. Conclusion

The fermentation process led to a reduction in the total quantified concentrations of hydroxybenzoic acids, procyanidins, and flavonols while increasing the contents of flavan-3-ols and hydroxycinnamic acids. The major differences in phenolic composition and certain sensory characteristics (astringency, sweet and sour tastes, pear and cooked pear odors) between the pear beverage samples followed the juice characteristics of different types of tested pear cultivars and breeding selections. In general, samples fermented with the *S. cerevisiae* strain had higher ethanol contents in comparison to the *T. delbrueckii* samples. However, the use of *T. delbrueckii* strains resulted in higher glycerol contents in the fermented pear beverages. Certain pear cultivars observed in the 'perry usage group', including 'Py10' and 'Sto', were separated from the rest of the selected pear cultivars due to their higher phenolic compositions, such as hydroxybenzoic acids, procyanidins, flavan-3-ols, hydroxycinnamic acids, and flavonols. The obtained alcoholic pear beverages with higher astringency and sourness were made from sour and astringent pear cultivars, whereas the sweet pear beverages were made from sweet dessert pear cultivars. Fermentation with different yeasts also affected the phenolic compositions of the final pear beverage products by increasing the concentrations of detected hydroxycinnamic acids as well as the total quantified residual sugar contents and the succinic acid contents. In addition, the use of *T. delbrueckii* resulted in higher intensities of cooked pear and floral odors, as well as a sweeter taste. Moreover, the contents of certain hydroxybenzoic acids, flavan-3-ols, procyanidins, hydroxycinnamic acids, and the main flavonols correlated with astringency. The pear cultivars in the 'perry usage group', such as 'Py10' and 'Sto', can be applied in pear beverage production, whereas certain cultivars of this study ('Py13' and 'Kru') may be more suitable as dessert pears in the 'dessert usage group'. Investigations concerning the volatile compounds of fermented pear beverages need to be carried out in future studies. Additionally, more studies are needed to further characterize the factors required for good quality 'perry', to define the compositional and sensory quality of the pear cultivars and to investigate the suitability of certain pear cultivars for commercial-scale alcoholic beverage processing. In general, the findings of this study promote the development and utilization of novel pear cultivars, as well as the use of non-*Saccharomyces* yeasts for the production of alcoholic beverages.

CRediT authorship contribution statement

Wenjia He: Methodology, Investigation, Formal analysis, Writing – original draft, Visualization, Data curation, Validation. **Ye Tian:** Methodology, Writing – review & editing. **Shuxun Liu:** Methodology, Writing – review & editing. **Laura Vaateri:** Investigation, Formal analysis. **Xueying Ma:** Writing – review & editing. **Tuuli Haikonen:** Resources, Writing – review & editing. **Baoru Yang:** Supervision, Project administration, Writing – review & editing. **Oskar Laaksonen:** Methodology, Writing – review & editing, Visualization, Supervision.

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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.136184.

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