Food Bioscience 62 (2024) 105447

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Impact of yeast selection on composition of vinegar fermented from pomace of a Finnish apple cultivar

Qizai Wang [a], Oskar Laaksonen [a], Elsa Xifre Pujol [a], Maarit Heinonen [b], Baoru Yang [a], Niina Kelanne [a,*]

[a] Food Sciences, Department of Life Technologies, University of Turku, FI-20014, Turku, Finland
[b] Natural Resources Institute Finland (Luke), Production Systems/Plant Genetics, Toivonlinnantie 518, FI-21500, Piikkiö, Finland

ARTICLE INFO

Keywords:
Apple pomace
Acetic acid fermentation
Saccharomyces cerevisiae
Torulaspora delbrueckii
Lachancea thermotolerans
Volatile compounds

ABSTRACT

Alcoholic beverages and vinegar products were produced using the pomace of a Finnish apple cultivar, and the influence of yeast strains on the non-volatile and volatile compound contents were studied. Apple pomace mashes were fermented with *Saccharomyces cerevisiae, Torulaspora delbrueckii*, and sequential inoculation with *Lachancea thermotolerans* and *S. cerevisiae* to produce alcoholic beverages. Commercial non-pasteurized vinegar was used as a starter to ferment alcoholic beverages into vinegars. Ethanol, acetic acid, sugars and organic acids were determined using GC-FID. A total of 63 volatile compounds were detected in the samples using HS-SPME-GC-MS. Alcoholic beverages fermented with the sequential inoculation had the highest total content of organic acids, and pure inoculation with *T. delbrueckii* resulted in the highest total content of sugars. Selection of different yeast strains did not alter the content of ethanol or acetic acid, but it significantly influenced the composition of volatile compounds in alcoholic beverages and vinegars. Compared to initial apple pomace mash, significant increases in the contents of ethyl esters (up to 4810-folds), higher alcohols (up to 24-folds) and volatile acids (up to 1853-folds) were observed in fermented products depending on the yeasts used. The main volatile compounds in vinegars prepared from *S. cerevisiae* fermented beverage were 2-phenylethanol, octanoic acid, and decanoic acid, from *T. delbrueckii* beverage 2-methylpropanoic acid and 3-methylbutanoic acid, and from sequential fermentation ethyl acetate, and ethyl 2-hydroxypropanoate. In conclusion, non-*Saccharomyces* yeasts have potential to produce more complex aromatic profile to fermented products derived from by-products of apple juice processing.

1. Introduction

Apples (*Malus domestica* Borkh) are a prominent fruit cultivated worldwide and rank the fourth most consumed fresh fruit globally (Zhang et al., 2021). Due to their versatility and chemical composition rich in phenolic compounds and other antioxidants, apples have a positive impact on the human health (Perussello et al., 2017). The global production of apples reached approximately 93 million metric tons in 2023 (FAOSTAT, 2023). While the majority of apples are consumed as fresh fruit, approximately 25% are processed into products, such as juice, cider and jam (Asif et al., 2024). During juice production, about 75% of the apple's weight is extracted as juice, leaving behind apple pomace (AP), a by-product that contributes to nearly 4 million tons of waste globally each year (Asif et al., 2024). AP accounts for roughly 25%–30% of the fresh apples, consisting primarily of apple peel and pulp (95%), along with seeds and stems (5%) (Shalini & Gupta, 2010). The residue is abundant in water and contains valuable natural compounds such as soluble sugar and phenols. Interestingly, AP has been found to contain even higher levels of bioactive substances compared to the juice itself (Shalini & Gupta, 2010). O'Shea et al. (2015) determined that 84.7% of the components of AP are carbohydrates and 54.2% are total sugars (mainly fructose and glucose). Therefore, AP is conducive to the growth and reproduction of microorganisms, making it an ideal matrix for vinegar production. Viroli et al. (2021) and Kalemba-Drożdż et al. (2020) successfully manufactured vinegar using apple peels, thus providing another alternative way to add commercial value to the side stream of apple juice processing.

Vinegar is produced through two consecutive fermentations: first, alcoholic fermentation with yeast (usually *Saccharomyces cerevisiae*), and then acetic fermentation with acetic acid bacteria (AAB, mainly

* Corresponding author.
E-mail address: niina.m.kelanne@utu.fi (N. Kelanne).

https://doi.org/10.1016/j.fbio.2024.105447
Received 13 August 2024; Received in revised form 6 November 2024; Accepted 8 November 2024
Available online 10 November 2024

Acetobacter pasteurianus and *Acetobacter polyoxogenes*; Luzón-Quintana et al., 2021). Fermentable sugars are converted into ethanol during alcoholic fermentation under anaerobic conditions, and ethanol is converted into acetic acid during acetic fermentation under aerobic conditions (Ho et al., 2017). Unpasteurized commercial vinegar, containing so called mother vinegar, can be used as a starter; alternatively, a pure culture of AAB can be employed to produce apple vinegar during the acetic fermentation process (Guiné et al., 2021). Kalemba-Drożdż et al. (2020) and Budak (2021) successfully used inoculation of commercial vinegar during acetic fermentation of apple peels or juice, respectively. Vinegar is rich in bioactive compounds, such as organic acids, amino acids, phenolics, and vitamins, that are beneficial for health (Chen et al., 2016; Xia et al., 2020). Antibacterial and anti-infection properties, control of blood glucose levels, regulation of lipid metabolism and weight loss have been related to organic acids present in vinegar (Chen et al., 2016). In addition, acetic acid in fruit vinegars has reduced blood glucose concentration after a starch-rich meal in healthy people (Johnston et al., 2009). Furthermore, vinegar has been observed to reduce fatigue, improve appetite, prevent osteoporosis, as well as to have anti-cancer, anti-inflammatory and immune-stimulatory effects (Kalaba et al., 2019).

The selection of yeast has an important role in determining the quality of vinegar (Ho et al., 2017). Yeasts can be split into two groups belonging to *Saccharomyces* and non-*Saccharomyces* yeasts, respectively. Traditionally, non-*Saccharomyces* yeast have been considered as spoilage yeasts in wine production since their fermenting results in compounds affecting negatively the quality of the beverages. However, many studies have shown importance of non-*Saccharomyces* yeasts for improving the sensory quality of wines (Morata et al., 2020). For instance, non-*Saccharomyces* species *Torulaspora delbrueckii* stands out as a promising non-*Saccharomyces* yeast for its capacity to yield different fruity aromas (Benito, 2018a; Lorenzini et al., 2019) and is appreciated in alcoholic beverages production for its remarkable capacity for production of succinic acid (Fernandes et al., 2021). *Lachancea thermotolerans* is attracting interest in alcoholic beverages industry due to its extraordinary ability to convert malic acid to lactic acid, which has led to an increased use of this yeast in winemaking and even sour beer production (Benito, 2018b; Vicente et al., 2021). However, *L. thermotolerans* has a low fermentation rate and thus low ethanol production, making sequential inoculation with conventional *Saccharomyces* yeast usually necessary (Porter et al., 2019).

The sensory quality of ciders and its consumers' acceptance is mainly determined by several volatile compounds that form the aroma profile (Perestrelo et al., 2019). These compounds are classified in various chemical classes, and the most important belong to higher alcohols, esters, volatile acids, terpenoids, aldehydes and ketones (Qin et al., 2018). According to He et al. (2021), the type of volatile compounds present in ciders depends on the apple variety, growing conditions, ripeness, and yeast strains responsible of the fermentation. In addition, the volatile composition of ciders differs in alcoholic beverages obtained with *Saccharomyces* and non-*Saccharomyces* yeast. In the case of vinegar, the final quality and consumer acceptance are also defined by the aroma and flavor derived from characteristic volatile compounds, including acetic acid (Jo et al., 2013; Mounir et al., 2018).

Vinegar is commonly consumed in Asian countries, with China alone consuming over 3.2 million liters of vinegar daily (Ho et al., 2017). In addition, apple vinegar is one of the most prevalent vinegar varieties in the European market, experiencing a rising demand and holding significant commercial value in the Scandinavian region. Therefore, it is important to prioritize the use of local ingredients. We have previously studied the roles of apple cultivars and yeast selection in cider production (He et al., 2021, 2022; Laaksonen et al., 2017), and we have completed various yeast fermentation studies (doctoral theses by He, 2022, Kelanne, 2021; Liu, 2020) in recent years. In this study, the aim was to make first alcoholic beverages (ABV) and then vinegar products from apple pomace, the side stream from apple juice production. Main emphasis was to characterize the effects of *Saccharomyces* and non-*Saccharomyces* strains, specifically comparing *S. cerevisiae* with *T. delbrueckii* and *L. thermotolerans* (sequential inoculation with *S. cerevisiae*), on the chemical compositions of the ABV and vinegars. The compositional investigations included analyses of volatile compounds using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS), and ethanol, acetic acid, sugars and organic acids using gas chromatography flame ionization detector (GC-FID). The findings form important knowledge and guidance to promote the valorization of the side streams from apple processing as a promising sustainable practice to increase the value of agricultural by-products and promoting the use of Finnish apple varieties.

2. Materials and methods

2.1. Standard compounds

Octanal, nonanal, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, butyl butanoate, butyl hexanoate, hexyl butanoate, ethyl dodecanoate, benzaldehyde, β-damascenone, ethyl decanoate, hexanoic acid, 1-heptanol, 1-octanol, 1-octen-3-ol, ethyl butanoate, 2-methylbutyl acetate, hexyl acetate, 2-methylpropyl acetate, 6-methylhept-5-en-2-one, tartaric acid, xylitol, quinic acid, L-malic acid, acetic acid, L-(+)-lactic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). DL-malic acid, ethyl hexanoate, butyl acetate, D-sorbitol were purchased from Fluka Chemicals (Neu Ulm, Bavaria Germany). D-(+)-fructose, D-(+)-glucose, and succinic acid were purchased from Merck (Darmstadt, Hesse, Germany). Citric acid and maleic acid were purchased from J.T. Baker Chemicals (Leuven, Flemish Brabant, Belgium). Tri-Sil® HTP reagent was purchased from Thermo Scientific (Bellefonte, PA, USA). Ethanol (≥99.5%) was purchased from ALTIA oy (Rajamäki, Uusimaa, Finland). All standards used in GC-MS analysis were a purity of ≥95%.

2.2. Preparation of the mash

Apples of Aleksanteri variety were harvested from Maaria's parish in the city of Turku in southwest Finland (60°27′ N, 22°16′ E) in September 2022. Apples were later juiced using a Kenwood Chef XL instrument with centrifugal juicer attachment (AT641, Kenwood Limited, Havant, Hampshire, United Kingdom). Before juicing, apples were washed with cold tap water, drained, and cut into small pieces. The AP (i.e. juice production residue with pomace, peels and seeds) was collected and transferred to sealable plastic bags and stored in a freezer at -21 °C for a maximum of 2 weeks. The frozen AP was weighted and mixed with Milli-Q water in a ratio of 1:5 and mashed using Bamix blender (Mettlen, Thurgau, Switzerland). The total soluble solids (TSS) of the apple mash was 2.2 °Brix. To assure a high enough final ethanol content (6%–10%), TSS was adjusted to 12.5 °Brix by adding solid glucose. Finally, the mixture was evenly transferred to six sterilized 1L storage bottles, two for inoculation with each yeast or yeast combination.

2.3. Preparation of alcoholic beverage

Three different active dry yeasts Lalvin Persy™ (*Saccharomyces cerevisiae*; SC), BIODIVATM TD291 (*Torulaspora delbrueckii*; TD), and LAKTIATM (*Lachancea thermotolerans*; LT) were provided by Lallemand Inc. (Montreal, QC, Canada) were mixed with nutritional broth Fermaid K (Lallemand Inc.), following dosage recommendations of the supplier. Approximately 4×10^9 CFU/mL of each yeast were inoculated. After the inoculation, each fermentation bottle was shaken vigorously to obtain even distribution of yeast in the mash. Bottles were closed with airlocks to obtain optimal anaerobic conditions for the yeasts. Fermentations were conducted in duplicates per yeast/yeast combination at room temperature (21 °C) in a dry and dark place. Fermentation dynamics was

monitored by calculating the weight loss of the bottles every day and by measuring °Brix value by a refractometer (HI-96801, Hanna Instruments, Bedford, Bedfordshire, United Kingdom) every week. The alcoholic fermentation was assumed to be ended, when the Brix value did not change over two days. The fermentation bottles were stirred carefully by hand every day during the whole fermentation period which lasted for 29 days.

2.4. Preparation of vinegar

Unpasteurized and unfiltered organic apple cider vinegar containing the AAB mother (Raw vibrant living, London, United Kingdom) was bought from a local store. It was directly plated in YPM agar and incubated at 30 °C for two days, to determine its AAB content. The vinegar contained approximately 2.6×10^7 CFU/mL of AAB. Vinegar was later added straight to the ABV at a concentration of 1:10, aiming for an inoculation of 10^6 CFU/mL of AAB.

The ABV was centrifuged at 10 000×*g*, for 10 min at 15 °C using Avanti JXN-26 centrifuge (Beckman Coulter, Indianapolis, IN, USA). The supernatants of each yeast were pooled for vinegar fermentation. ABV (90 mL) and commercial vinegar (10 mL) were transferred to sterilized 100 mL storage bottles. Each bottle was covered with a piece of gauze to avoid contamination while maintaining an aerobic environment. Vinegar fermentations were conducted in triplicates per yeast fermentation at room temperature in a dry and dark place and stirred every day for 27 days. Fermentations were monitored by measuring pH weekly with an inoLab pH Level 1 (WTW, Weilheim, Bavaria, Germany) pH-meter. The endpoint of the acetic fermentation was assumed to be reached when the pH value did not notable change in two days.

2.5. Analysis of ethanol and acetic acid

A gas chromatography (Shimadzu Nexis GC-2030; Shimadzu Corp Kyoto, Japan) with an autoinjector (A9OC-20i), an autosampler (AOC-20s), and a flame ionization detector (GC-FID) was used to identify and quantify ethanol and acetic acid concentrations. The samples were filtered with a regenerated cellulose (RC) 0.2 μm hydrophilic filter and placed in 1.5 mL autosampler vials. A polar HP-INNOWax (30 m × 0.25 mm x 0.25 μm, Hewlett-Packard, Palo Alto, CA, USA) column was used to separate the compounds. The injection temperature was set to 220 °C. The injection volume was 0.2 μL, with a split ratio of 25. The oven temperature was set to 40 °C (hold for 5 min), followed by an increase of 40 °C/min to 240 °C. The carrier gas was helium with a flow of 1.6 mL/min and the linear velocity constant was set at 35 cm/s. FID temperature was set to 250 °C. LabSolutions software (v. 5.106 SP1, Shimadzu Corp., Kyoto, Japan) was used to operate the instrument and for the post run analysis. External standards of ethanol and acetic acid, at concentrations of 2, 4, 6, 8, and 10%, were also analyzed to enable identification and quantification of these compounds in the samples.

2.6. Analysis of sugars and organic acids

The sugars and organic acids were analyzed from the ABV and vinegar samples with a gas chromatograph (GC-2010Plus, Shimadzu Corp.) equipped with a flame ionization detector (FID) and an AOC-20 autosampler using method described previously with slight modifications (Kelanne et al., 2019). Briefly, sugars and organic acids were analyzed as trimethylsilyl (TMS) derivatives. External standards (sorbitol, tartaric acid, malic acid, ascorbic acid, lactic acid, maleic acid, citric acid and succinic acid, all 5 g/L) were used to identify and quantify of the main sugars and organic acids. Xylitol and shikimic acid (both 5 g/L) were used as internal standards for quantification of sugars and organic acids, respectively. Two hundred and fifty μL of sample and both internal standards were mixed, and the mixture was diluted to final volume of 5 mL with ultra-pure water (Millipore Corp., Darmstadt, Hesse, Germany). The sample was then filtered with a RC syringe filter (0.45 μm). A portion of 300 μL of the filtrate was pipetted to an autosampler vial and evaporated to dryness at 50 °C under a nitrogen flow. The samples were stored in the desiccator until analysis or at least overnight. Five hundred μL of Tri-Sil reagent (hexamethyldisilazane:trimethylchlorosilane:pyridine, 2:1:10, Thermo Scientific, Pierce Biotechnology, Rockford, IL, USA) was added to the samples for trimethylsilyl (TMS) derivatization; the sample was then vortexed vigorously for 5 min and incubated for 30 min at 60 °C. Then samples were cooled to room temperature before analysis. The analyses of the derived samples were carried out with Supelco Simplicity-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm d_f, Supelco, Bellefonte, PA, USA). Aliquot of each sample (1 μL) was automatically injected using a split/splitless injector with a split ratio of 1:15. Helium served as the carrier gas at a flow rate of 1.9 mL/min. The injector temperature was set to 210 °C and the detector temperature to 290 °C. The column temperature program started at 150 °C for 2 min, then increased to 210 °C at 4 °C/min, and finally to 275 °C at 40 °C/min, holding at 275 °C for 5 min.

2.7. Analysis of volatile compounds

Headspace solid phase microextraction coupled with gas chromatography and mass spectrometry (HS-SPME-GC-MS) was used to identify and quantify volatile compounds, using the methodology described by Vicente et al. (2023). Briefly, 0.2 g of sodium chloride, 2 mL of sample, and 10 μL of 4-methyl-2-pentanol solution (802 μg/mL in methanol) as an internal standard were added to a 20 mL vial. Each sample was prepared in triplicate. The volatile compounds were extracted from the headspace with a 2 cm DVB/CAR/PDMS fiber (50/30 μm, Supelco, Bellefonte, PA, USA) at 45 °C for 30 min after 10 min of incubation. The fiber was conditioned at 250 °C prior to the sample extraction. After the extraction, the SPME fiber was immediately transferred to the injection port of a Trace 1310 gas chromatograph equipped with a TSQ 7000 EVO mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) to be thermally desorbed in splitless mode at 240 °C for 3 min. A DB-WAX polar capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was used to separate the volatile compounds of the samples. Helium was used as the carrier gas at a flow rate of 1.6 mL/min. The initial column temperature was set to 50 °C and held for 3 min. Afterwards, the temperature was increased to 220 °C at a rate of 5 °C/min and held at 220 °C for 8 min. Mass spectra were detected in electron impact (EI) mode at 70 eV, with a scan range from *m/z* 33 to *m/z* 300. The MS transfer line and the ionization source temperatures were 220 and 240 °C, respectively.

The identification of the volatiles was done by comparing the obtained mass spectra with the standard NIST library and comparing their retention indices (RI) reported in NIST database. The calculation of the RI was done by co-injecting an alkane mixture (C7-C21, Sigma-Aldrich, St. Louis, MO, USA). Moreover, the identification of a selected number of volatile compounds was confirmed by comparing the RI and mass spectra with those of the authentic reference compounds. The semi-quantification of individual volatile compounds was conducted by comparing their areas at specific retention times (Supplementary Table 1) to peak area of the internal standard (Liu et al., 2019), using the following equation:

$$C\ (\mu g / L) = \frac{Ac}{As}\ C_S\ (\mu g / L)$$

Where C = relative concentration of analyte, A_C = peak area of analyte, A_S = peak area of internal standard, C_S = final concentration of internal standard in samples.

2.8. Statistical analysis

The semi-quantitative data were used for statistical comparison and multivariate analysis to measure the ABV and vinegar samples produced

from AP with different yeast strains. One-way ANOVA test and Tukey's test were used to evaluate the chemical composition differences using IBM SPSS Statistics 29.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$. Multivariate models were created with SIMCA (version 18, Umetrics, Umeå, Västerbotten, Sweden). Principal components analysis (PCA) was carried out to study the sample groupings and correlations among volatile profiles among ABV and vinegar samples.

3. Results and discussion

3.1. Fermentation kinetics

The general fermentation parameters, which include ethanol content (v/v, %), total soluble solids (TSS) expressed in °Brix, and pH, of AP mash and its corresponding ABV and vinegar samples, are shown in Table 1 and Fig. 1.

Table 1
Chemical compositions of apple pomace mash and its corresponding alcoholic beverage and vinegar samples.

Sample	Mash	Alcoholic beverage samples			Vinegar samples		
		SC	LT + SC	TD	SC	LT + SC	TD
TSS (°Brix)	12.60 ± 0.00	3.45 ± 0.07	3.50 ± 0.00	3.65 ± 0.35	/	/	/
pH	/	3.29 ± 0.00	3.29 ± 0.00	3.30 ± 0.00	2.74 ± 0.02	2.78 ± 0.02	2.73 ± 0.01
Ethanol content (v/v, %)	0.00 ± 0.00 b	6.90 ± 0.27 a	7.02 ± 0.41 a	6.87 ± 0.63 a	0.41 ± 0.14 b	0.33 ± 0.25 b	0.19 ± 0.14 b
Acetic acid (%)	0.15 ± 0.00 b	0.47 ± 0.07 b	0.47 ± 0.04 b	0.45 ± 0.00 b	2.84 ± 0.02 a	2.81 ± 0.82 a	3.53 ± 0.22 a
Sorbitol (mg/L)	/	1.67 ± 0.20	2.10 ± 0.02	1.66 ± 0.06	1.63 ± 0.24	1.49 ± 0.12	1.67 ± 0.25
Glucose (mg/L)	/	ND	ND	1.88 ± 2.66	1.25 ± 0.23	0.99 ± 1.41	2.60 ± 0.71
Fructose (mg/L)	/	ND	0.81 ± 0.33	3.26 ± 0.69	ND	0.29 ± 0.41	3.38 ± 1.77
Total sugars (mg/L)	/	1.67 ± 0.20	2.90 ± 0.31	6.80 ± 3.29	2.88 ± 0.01	2.77 ± 1.12	7.65 ± 0.81
Lactic acid (mg/L)	/	ND	2.86 ± 0.42	ND	ND	2.07 ± 0.27	ND
Maleic acid (mg/L)	/	24.65 ± 2.62	46.08 ± 1.30	20.46 ± 4.14	23.29 ± 3.67	34.31 ± 1.63	20.92 ± 1.02
Succinic acid (mg/L)	/	3.27 ± 0.11	5.30 ± 0.14	3.36 ± 0.14	3.14 ± 0.30	3.88 ± 0.27	3.38 ± 0.59
Malic acid (mg/L)	/	5.68 ± 0.34	4.50 ± 0.10	4.33 ± 0.19	5.62 ± 0.58	3.29 ± 0.29	4.34 ± 0.37
Quinic acid (mg/L)	/	0.77 ± 0.12	0.80 ± 0.01	0.46 ± 0.17	0.73 ± 0.19	0.58 ± 0.04	0.57 ± 0.06
Total organic acids (mg/L)	/	35.26 ± 2.04	59.54 ± 1.96	28.61 ± 4.36	32.83 ± 4.80	44.14 ± 1.96	29.20 ± 0.01

Results represent the mean ± standard deviation. Mash samples in triplicate, alcoholic beverage samples in duplicate and vinegar samples in six replicates (2 biological replicates × 3 analytical replicates). Statistically significant differences of ethanol and acetic acid contents between yeasts within each sample type, alcoholic beverage and vinegar, are shown with lower case letters a-b (ANOVA with Tukey's test). ND: not detected.; SC: *S. cerevisiae*; LT + SC: *L. thermotolerans* + *S. cerevisiae*, TD: *T. delbrueckii*.

3.1.1. Determination of TSS and ethanol concentrations after alcoholic fermentation

The final °Brix of ABV fermented with SC, TD, and LT + SC was 3.45, 3.65, and 3.50 (Table 1). These results aligned with the findings of Budak (2021) and Tocci et al. (2023), who reported 3.60 °Brix in apple cider fermented with *S. cerevisiae* and approximately 4.53 °Brix in apple cider fermented with *T. delbrueckii*.

While a significant decrease in TSS contents was observed after alcoholic fermentation, the concentration of ethanol increased considerably (Fig. 1). The ethanol concentration of final ABV ranged from 6.87% (TD) to 7.02% (LT + SC) (Table 1). This was in accordance with other ciders fermented with the same yeast species (Budak, 2021; Lorenzini et al., 2019; Tocci et al., 2023; Wei et al., 2020). This phenomenon is due to yeast metabolism, where glucose from the raw material was oxidized and converted into ethanol and other compounds, e.g. acetic acid, as described by Desiderio Estela Escalante (2019). A slightly higher level of ethanol was observed in the fermentation with LT + SC compared to the other two yeasts. This phenomenon may be related to the "make-accumulate-consume" fermentation performance of *S. cerevisiae*, as described by Parapouli et al. (2020). Hence, the sequential inoculation of non-*Saccharomyces* and *Saccharomyces* yeasts is viewed as a promising method for lower ethanol content in fermented beverages. One-way ANOVA analysis determined that the differences in the final ethanol concentration among yeast strains were not statistically significant (Table 1). Nevertheless, ethanol production with LT + SC was distinct from the rest, reaching a peak in its concentration and then decreasing.

3.1.2. Acetic acid concentrations after acetic fermentation

During acetic fermentation, ethanol concentration and pH values decreased, while acetic acid concentration increased (Table 1). These changes were the results of AAB metabolism. Vinegar samples originally fermented with TD had the highest acetic acid and lowest ethanol concentrations compared to other two yeasts. Nevertheless, the differences in the final ethanol and acetic acid concentration among yeast species were not significant, according to the results of a one-way ANOVA analysis (Table 1).

The acetic acid concentration of the final vinegars ranged from 2.81% to 3.53% (Table 1). The obtained acetic acid concentrations were lower than the result of apple peel vinegars reported by Viroli et al. (2021), though the pH and ethanol concentrations were similar to those vinegars made from apple juice and apple peel in the literature (Budak, 2021; Kalemba-Drożdż et al., 2020; Viroli et al., 2021). Mas et al. (2014) stated that a low concentration of ethanol (usually 0.5%–1%) is needed to avoid AAB from completely transforming acetic acid to CO_2 and H_2O through the tricarboxylic acid cycle. However, too low amounts (0.19%–0.41%) were observed in this study. In this sense, a higher °Brix would have been necessary to obtain higher ethanol concentrations, which would have been transformed into higher acetic acid concentration and residual ethanol content in the final product.

3.2. Sugars and organic acids in alcoholic beverage and vinegar

Fructose is the main sugar present in ABV and the corresponding vinegar, followed by sorbitol and glucose (Table 1). SC, TD and LT are reported to prefer consuming glucose over fructose (Benito, 2018a; Vicente et al., 2023), resulting in considerable amounts of fructose remaining in ABV. The samples fermented with TD had the highest total sugar level. This suggested that TD yeast by itself is unable to fully use the high sugar content in AP mash, leading to a low conversion rate of sugar to ethanol (Liu et al., 2018). This corresponded with the observation that ABV fermented with TD had the lowest ethanol content.

Maleic acid was the most abundant organic acid in all ABV and vinegar samples, accounting for 70%–78% of the total organic acid content (Table 1), whereas malic acid is the main organic acid in apples, accounting for approximately 90% of the total organic acid content

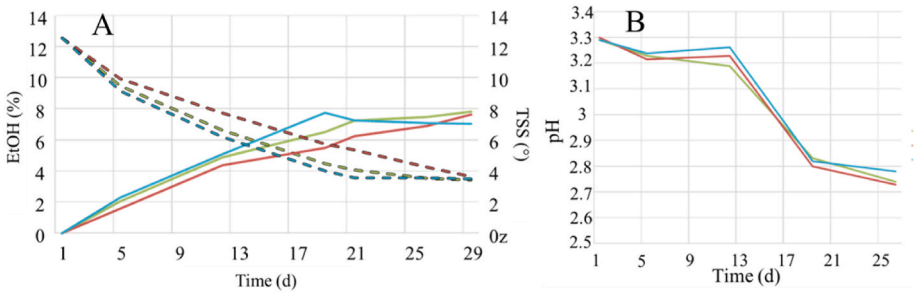


Fig. 1. Fermentation kinetics during A) alcoholic fermentations, and B) vinegar fermentations presented as changes of A) ethanol and TSS contents, and as B) decrease of pH. Line colours (both solid and dashed) represent each yeast used to ferment alcoholic beverages: green *S. cerevisiae*, red *T. delbrueckii*, and blue *L. thermotolerance* + *S. cerevisiae*. In A, solid line represents ethanol content and dashed line total soluble solids (TSS). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Aprea et al., 2017; Lee & Wrolstad, 1988). ABV fermented with LT + SC had lower content of malic acid than SC samples. This might have resulted from the ability of LT to use malic acid present in AP mash to produce lactic acid (Benito, 2018b; Vicente et al., 2021). This also could explain why only LT + SC samples had lactic acid. According to Liu et al. (2018), reducing the content of malic acid may have potential benefits, such as decreasing the harsh 'green apple sourness' acidity and puckering astringency. Co-fermentations LT + SC increased the final acidity compared to sole inoculation of SC, mainly due to the significant increase of maleic acid. The results showed that LT + SC samples had highest total acid level. In addition, trace amount of citric acid was observed in all fermented samples.

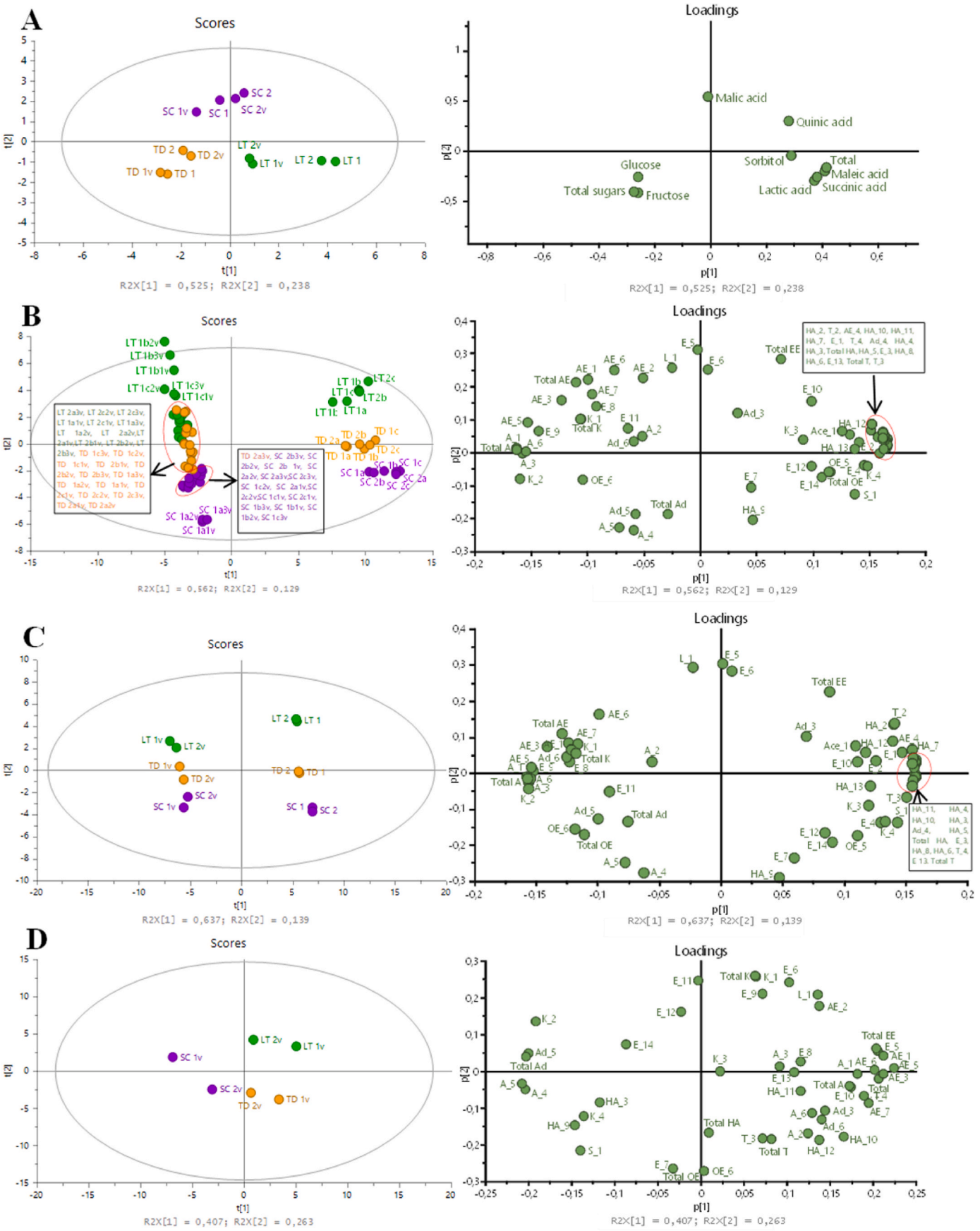
Principal component analysis (PCA) was used to illustrate the differences of sugars and organic acids between the ABV and vinegar samples (analytical replication are averaged to biological replicates) fermented with three different yeasts (Fig. 2A). PC-1 explained 52.5% of the total variance, whereas PC-2 explained 23.8% of the variance. Surprisingly, ABV and vinegar samples fermented with certain yeast were clustered together, indicating no significant changes were caused by the vinegar fermentation. However, clear difference was observed between the samples by yeasts used: LT + SC and TD were separated from each other on PC-1, and SC samples were clearly separated from TD and LT + SC samples on PC-2. Malic acid and quinic acid were the only compounds positively correlating with SC samples, and negatively correlating with other samples. Succinic acid, lactic acid, maleic acid, total acid content, and sorbitol were positively correlating with LT + SC samples. Glucose, fructose and total sugar content were only positively correlating with TD samples. These results indicate that yeast strains play significant role determining the composition and quality of ABV and vinegars fermented from AP.

3.3. Volatile compounds in mash, alcoholic beverage and vinegar

A total of 63 volatile compounds were identified in mash and fermented samples by the DB-WAX Column, including 27 esters (14 ethyl esters, EE; seven acetate esters, AE; six other esters OE), 13 higher alcohols (HA), six volatile acids (A), six aldehydes (Ad), four terpenoids (T), four ketones (K), one lactone (L), one acetal (Ace) and one sulfur compound (S) (Table 2). Semi-quantitative data of all identified volatile compounds are given in Supplementary Table 1. Thirty-two volatile compounds were identified in the mash, of which eight compounds became undetectable after alcoholic fermentations. Regarding the ABV, 49 volatile compounds were identified while 6 compounds were not observed in the original mash but appeared after alcoholic fermentation and became undetectable after acetic fermentation. Finally, 39 different volatile compounds were identified in the vinegars, of which three compounds (ethyl tetradecanoate, E_9; 3-methylbutanoic acid, A_6; and benzaldehyde, Ad_5) were only found in vinegars. In addition, concentrations of some compounds, such as hexyl hexanoate (OE_5), 1-hexanol (HA_3) and α-farnesene (T_3), which were present in high contents in original mash (Balázs et al., 2012; Ferreira et al., 2009; Yang et al., 2021) but were reduced in vinegars by the fermentations (Supplementary Table 1). Most likely these compounds were transformed into other compounds, e.g. α-farnesene may have been subsequently oxidized to conjugated trienes (Fraternale et al., 2011).

Esters, including ethyl esters, acetate esters, and other esters, were the predominant volatile compounds class in both ABV and vinegar samples (Table 3). While some esters were present in the mash initially, the majority were generated from the esterification reaction (Madrera et al., 2015; Ye et al., 2014). Ethyl acetate (E_5) was the primary ester in both types of samples (Supplementary Table 1), and possibly contributing to pleasant flavors, such as *pineapple, fruity, pungent,* and *varnish* (He et al., 2021). The participation of *S. cerevisiae* in sequential fermentation significantly increased the production of ethyl acetate (E_5) compared to *S. cerevisiae* in pure fermentation. The result in Supplementary Table 1 showed that LT + SC fermentation yielded approximately 3-fold higher content of ethyl acetate (E_5) compared to SC ABV. The total ethyl ester content was also statistically significantly higher in LT + SC ABV compared to the pure fermentation with TD or SC. This increase may be attributed to the fact that SC typically produces higher level of esters than non-*Saccharomyces* yeasts. Therefore, sequential inoculations of SC with non-*Saccharomyces* yeasts increased ester formation. Similar results were previously observed by Liu et al. (2019) and Wang et al. (2023). Both studies compared pure fermentations and sequential fermentation involving *S. cerevisiae* and non-*Saccharomyces* yeast strains (*S. pombe and/or T. delbrueckii*), either in bilberry wines or blueberry fermented beverage. They observed that sequential inoculations of *S. cerevisiae* with non-*Saccharomyces* yeasts increased the formation of esters. Furthermore in our study, among minor esters, ethyl octanoate (E_4), ethyl hexanoate (E_13), 3-methylbutyl acetate (AE_6) and diethyl succinate (OE_6) were the predominant volatile compounds in ABV, while in vinegar, 2-phenylethyl acetate (AE_5), 2-methylbutyl acetate (AE_3), 3-methylbutyl acetate (AE_6) and diethyl succinate (OE_6) were more prevalent.

Higher alcohols constituted the most abundant volatile group in ABV in terms of concentration (Table 3). While higher alcohols serve as important precursors for esters, an excess amount may have negatively impact on product quality, given the inhibitory effect of ethanol on yeast growth and viability (He et al., 2021). Most of the higher alcohols were produced during alcoholic fermentation with concentrations of 20–25 times higher in ABV compared to those in the initial AP mash samples. ABV fermented with TD showed the highest content of higher alcohols, surpassing that in the original mash by 25 times. 3-Methyl-1-butanol (HA_11) emerged as the predominant higher alcohol formed by deamination and decarboxylation reactions of amino acids, a finding consistent with findings in other apple ciders, contributing to *whiskey* and *malt* aroma (Fan et al., 2011; Qin et al., 2018; Villire et al., 2012; Xu et al., 2007). (2E)-2-hexen-1-ol (HA_1) was observed only in the mash samples



(caption on next page)

Fig. 2. Principal component analysis (PCA) models using contents of sugars, organic acids and volatile compounds to explain the differences between alcoholic beverages and vinegars with different yeasts. A) PCA using contents of sugars and organic acids (n = 9), alcoholic beverages (n = 6, averaged to 2 biological replicates) and vinegars (n = 6, averaged to 2 biological replicates) fermented with different yeasts. B) PCA using contents of volatile compounds (n = 63) to explain the differences between alcoholic beverages (n = 18 = 3 yeasts × 2 biological replicates × 3 analytical replicates) and vinegars (n = 54, all replications) with different yeasts. C) PCA using contents of volatile compounds (n = 63) to explain the differences between alcoholic beverages (n = 6, averaged to 2 biological replicates) and vinegars (n = 6, averaged to 2 biological replicates) with different yeasts. D) PCA using contents of volatile compounds (n = 63) to explain the differences in vinegars (n = 6, averaged to 2 biological replicates) originally fermented with different yeasts. SC is for *S. cerevisiae* (purple), TD for *T. delbrueckii* (yellow), and LT for *L. thermotolerans* + *S. cerevisiae* (green), v vinegar. The abbreviations of volatile compounds refer to those in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and became undetectable after alcoholic fermentation. Similar result was reported by Kelanne et al. (2022), who fermented black currants with *S. cerevisiae* or non-*Saccharomyces* yeasts (*T. delbrueckii, S. bayanus, M. pulcherrima* and *M. fructicola*). They only observed (2E)-2-hexen-1-ol (HA_1) in juice samples, and it became undetectable after fermentation. 1-Hexanol (HA_3), 2-methyl-1-butanol (HA_10) and 2-methyl-1-propanol (HA_12), which showed relatively high concentrations in all ABV, decreased during acetic fermentation, with their concentrations dependent on the yeast strains used. 1-Butanol (HA_2) and 1-hexanol (HA_3) are the primary alcohols detected in apples. 1-Butanol increases the apple aroma with its sweet scent, while conversely, 1-hexanol can mask the typical apple scent with an undesirable earthy smell, thereby detracting from the overall aroma (Yang et al., 2021). This study observed that all ABV samples had both 1-hexanol and 1-butanol, while only vinegar samples originally fermented with SC showed 1-hexanol.

Six volatile acids, namely acetic acid (A_1), 2-methylpropanoic acid (A_2), hexanoic acid (A_3), octanoic acid (A_4), decanoic acid (A_5), and 3-methylbutanoic acid (A_6), were identified in the samples. Volatile acids play an important role in the aromatic complexity of apple ciders, contributing to *vinegar-like, fruity, cheese* and *rancid* aromas (Qin et al., 2018). Acetic acid (A_1), known for its *pungent acidic* and *vinegar-like* odor, was produced by the fermentation of ethanol and was present in highest concentrations across all vinegar samples (Supplementary Table 1). The highest total volatile acid content was observed in vinegars originally fermented with TD. 3-Methylbutanoic acid (A_6) was exclusive to vinegar samples and was not detected in either the mash or ABV. All volatile acid contents increased during acetic fermentation.

Six aldehydes, namely hexanal (Ad_1), (2E)-2-hexenal (Ad_2), octanal (Ad_3), nonanal (Ad_4), benzaldehyde (Ad_5), and dodecanal (Ad_6), were detected in the samples. Hexanal and (2E)-2-hexenal were the most abundant aldehydes in this study, exclusively present in the mash samples. Hexanal typically plays a significant role in imparting the distinctive sweet, fish-like odors characteristic of apples, while (2E)-2-hexenal may add green, grassy attribute to the apple flavor profile (Yang et al., 2021). Furthermore, aldehydes presented in the mash were most likely reduced to their corresponding higher alcohols during fermentation (Kelanne et al., 2022).

The only sulfur compound identified was 3-(methylthio)-1-propanol (S_1), which arises from the breakdown of methionine during fermentation (Xu et al., 2007). This compound often contributes to undesirable flavors in ABV. Similar finding was reported by Liu et al. (2020), who investigated volatile compound profile during bilberry juice fermentation with nine non-*Saccharomyces* yeasts (including *T. delbrueckii* and *L. thermotolerans*) and reported 3-(methylthio)-1-propanol as the only sulfur compound detected in the bilberry ABV.

To determine patterns of the identified compounds of the samples, principal component analysis (PCA) was constructed using the initial AP mashes (n = 3) and the different fermentation products (ABV n = 18, vinegars n = 54) as samples and volatile compounds (n = 63) as variables (Supplementary Fig. 1). PC-1 explained 43.5% of the total variance, whereas PC-2 explained 26.8% of the variance. The original mash samples were clearly separated from the ABV and the vinegar samples. Furthermore, ABV and vinegar samples were separated from each other on PC-1 and clear product clusters were seen. Therefore, in the following analysis aiming for comparing the volatile profiles among the fermented products, we excluded the mash samples.

Two PCAs (Fig. 2 B&C) were used to explain the differences between ABV and vinegars with different yeasts. Fig. 2B comprised all data (18 ABV, 54 vinegar samples and 63 identified volatile compounds), whereas Fig. 2C comprised biological duplicates of each yeast (6 ABV, 6 vinegar samples and 63 identified volatile compounds). The first two PCs explained 69.1% (Figs. 2B) and 77.6% (Fig. 2C) of the data variance. PC-1 clearly separated the ABV samples from the vinegar samples on both PCAs. In addition, clear clusters were formed depending on the inoculated yeast on PC-2 (Fig. 2 B&C). Fig. 2C shows that ethyl octanoate (E_4), ethyl 2-hydroxy-4-methylpentanoate (E_7), ethyl dodecanoate (E_12), ethyl decanoate (E_14), hexyl hexanoate (OE_5), 6-methylhept-5-en-2-one (K_4), and 3-(methylsulfanyl)-1-propanol (S_1) were positively correlating with SC ABV samples. Ethyl heptanoate (E3), 1-octanol (HA_5), 1-decanol (HA_6), 1-octen-3-ol (HA_8), and β-damascenone (T_4) were positively correlating with TD ABV samples. Ethyl esters, especially ethyl acetate (E_5) and ethyl 2-hydroxypropanoate (E_6) were positively correlating to LT + SC samples. As mentioned before, LT has an ability of producing lactic acid (Vilela, 2018) and ethyl 2-hydroxypropanoate (E_6) is synthesized from ethanol and lactic acid (Delgado et al., 2010). Therefore, the presence of this volatile compound in the samples fermented with LT + SC could be explained by the metabolism of LT. The odor and flavor of this compound has been described as *sweet, fruity, creamy, acidic* and *caramellike* (Mosciano et al., 1993), which might be appreciated in both ABV and vinegar.

Fig. 2C also shows that total content of acetates (AE) was strongly positively correlated to propyl acetate (AE_1), 2-methylbutyl acetate (AE_3) and 2-methylpropyl acetate (AE_7). These compounds have been described as having a *floral* and *fruity* odor, which may be perceived as being pleasant in fermented apple beverages (He et al., 2021). The total content of other esters (OE) showed a strong positive correlation with the content of diethyl succinate (OE_6). The odor of this compound has been described as *fruity, melon,* and *wine fruity* (Liu et al., 2019; Madrera et al., 2015). The total content of higher alcohols (HA) showed positive correlations with 1-hexanol (HA_3), 1-heptanol (HA_4), 1-octanol (HA_5), 1-decanol (HA_6), 1-octen-3-ol (HA_8), 2-methyl-1-butanol (HA_10), and 3-methyl-1-butanol (HA_11). Among them, 1-octen-3-ol (HA_8) is a flavor compound used in food industry, due to its distinctive mushroom aroma (Madrera et al., 2015). 2-Methyl-1-butanol (HA_10) and 3-methyl-1-butanol (HA_11) are degradation products of isoleucine and leucine, respectively (Waterhouse et al., 2016). The total content of volatile acids (A) was positively correlated with acetic acid (A_1), hexanoic acid (A_3) and 3-methylbutanoic acid (A_6). However, high contents of straight-chain fatty acids (A_1 and A_3) may have a negative impact on the sensory properties (Kelanne et al., 2022). The total content of aldehydes (Ad) was positively correlated to the level of benzaldehyde (Ad_5). The odor of this compound has been described as *roasted, almond* and *cherry* (He et al., 2021; Liu et al., 2019). The total content of terpenoids (T) was positively correlated to the level of α-farnesene (T_3) and β-damascenone (T_4). During alcoholic fermentation, a significant increase in β-damascenone was observed, likely due to the hydrolysis of glycosylated precursors in the raw material. β-Damascenone is commonly used in industry for its distinctive *rose-like* aroma (Madrera et al., 2015). The total content of ketones (K) was positively correlated with 3-hydroxy-2-butanone (K_1), one of the potential contributors to the unpleasant fatty odor, as the compound has been described as *fatty, buttery,* and *creamy* (Liu et al., 2020).

Table 2
Identification of volatile compounds by HS-SPME-GC-MS in apple pomace mash, alcoholic beverage and vinegar samples.

Peak number[a]	Compounds	Abbreviations	RI		Presence				Identification[d]	Odor descriptor[e]
			Measured[b]	NIST[c]	Mash	ABS (SC/LT + SC/TD)	Reference vinegar	Vinegar samples (SC/LT + SC/TD)		
Ethyl esters										
1	Ethyl butanoate	E_1	1037	1036 ± 8	/	x	/	/	MS, LRI, STD	fruity[f]
2	Ethyl 2-methylbutanoate	E_2	1054	1052 ± 8	/	x	x	/	MS, LRI, STD	fruity[f]
3	Ethyl heptanoate	E_3	1336	1331 ± 8	/	x	/	/	MS, LRI	fruity[g]
4	Ethyl octanoate	E_4	1438	1435 ± 7	/	x	/	/	MS, LRI	sweet, brandy, fruity fat[f,g,h]
5	Ethyl acetate	E_5	889	888 ± 9	/	x	x	x	MS, LRI	pineapple, fruity, pungent, varnish[f,g]
6	Ethyl 2-hydroxypropanoate	E_6	1351	1347 ± 9	/	x	/	x	MS, LRI	fruity, buttery[g]
7	Ethyl 2-hydroxy-4-methylpentanoate	E_7	1552	1547 ± 1	/	x	/	x	MS, LRI	black currant, fruit[i]
8	Ethyl phenylacetate	E_8	1800	1783 ± 10	/	x	/	x	MS, LRI	fruity sweet[h]
9	Ethyl tetradecanoate	E_9	2058	2050 ± 12	/	/	x	x	MS, LRI	ether[h]
10	Ethyl 3-methylbutanoate	E_10	1069	1068 ± 8	/	x	x	x	MS, LRI, STD	fruity, apple[g]
11	Ethyl hexadecanoate	E_11	2254	2251 ± 10	/	x	x	x	MS, LRI	fat[h]
12	Ethyl dodecanoate	E_12	1851	1843 ± 8	/	x	x	x	MS, LRI, STD	brandy, fruity, grape, Leaves[g,h]
13	Ethyl hexanoate	E_13	1235	1233 ± 9	/	x	x	x	MS, LRI, STD	fruity, green apple, banana, brandy[f,g]
14	Ethyl decanoate	E_14	1643	1639 ± 8	x	x	x	x	MS, LRI, STD	fruity, brandy, burnt, grape [f,g,h]
Acetate esters										
15	Propyl acetate	AE_1	975	973 ± 11	x	/	x	x	MS, LRI	celery, floral, pear[f]
16	Butyl acetate	AE_2	1073	1074 ± 8	x	/	x	/ x /	MS, LRI, STD	apple, fruit, pungent[f]
17	2-Methylbutyl acetate	AE_3	1121	1125 ± 9	x	x	x	x	MS, LRI, STD	fruit[k]
18	Hexyl acetate	AE_4	1274	1273 ± 7	x	x	x	/	MS, LRI, STD	fruity, herbaceous[f,h]
19	2-Phenylethyl acetate	AE_5	1830	1813 ± 15	/	x	x	x	MS, LRI	flowery, fruit, Roses honey[f,g,h]
20	3-Methylbutyl acetate	AE_6	1122	1123 ± 8	/	x	x	x	MS, LRI	banana, fruity, sweet, apple[f,g]
21	2-Methylpropyl acetate	AE_7	1015	1012 ± 8	/	/ x x	x	x	MS, LRI, STD	apple, banana, floral[f]
Other esters										
22	Butyl butanoate	OE_1	1220	1220 ± 8	x	/	/	/	MS, LRI, STD	floral[f]
23	Butyl hexanoate	OE_2	1416	1407 ± 8	x	/	/	/	MS, LRI, STD	fruity, grass, green[f]
24	Hexyl butanoate	OE_3	1419	1414 ± 8	x	/	/	/	MS, LRI, STD	fruity, apple, fresh[f]
25	Hexyl 2-methylbutanoate	OE_4	1431	1433 ± 5	x	/	/	/	MS, LRI	Strawberry[f,h]
26	Hexyl hexanoate	OE_5	1615	1605 ± 8	x	x / x	/	/	MS, LRI	apple, fruity, orange peel peach[f,h,j]
27	Diethyl succinate	OE_6	1684	1681 ± 9	/	x	/	x	MS, LRI	fruity, melon, wine fruity[g,h]
Higher alcohols										
28	(2E)-2-hexen-1-ol	HA_1	1412	1406 ± 8	x	/	/	/	MS, LRI	grass[f]
29	1-Butanol	HA_2	1153	1142 ± 11	x	x	x	/	MS, LRI	medicine, fruit, alcohol[f,g]
30	1-Hexanol	HA_3	1360	1355 ± 7	x	x	x	x / /	MS, LRI	floral, green, herbaceous[h,j]
31	1-Heptanol	HA_4	1462	1453 ± 9	x	x	/	/	MS, LRI, STD	oily[f,g]
32	1-Octanol	HA_5	1564	1557 ± 8	x	x	/	/	MS, LRI, STD	chemical, metal, lemon, oily fatty[f,g,h]

(*continued on next page*)

Table 2 (*continued*)

Peak number[a]	Compounds	Abbreviations	RI		Presence				Identification[d]	Odor descriptor[e]
			Measured[b]	NIST[c]	Mash	ABS (SC/LT + SC/TD)	Reference vinegar	Vinegar samples (SC/LT + SC/TD)		
33	1-Decanol	HA_6	1771	1760 ± 9	x	x	/	/	MS, LRI	fat[h]
34	2-Ethyl-1-hexanol	HA_7	1494	1491 ± 5	x	x	x	/	MS, LRI	citrus, green, rose[f,g]
35	1-Octen-3-ol	HA_8	1454	1450 ± 7	x	x	/	/	MS, LRI, STD	mushroom[h,j]
36	2-Phenylethanol	HA_9	1932	1907 ± 15	x	x	x	x	MS, LRI	Honey, rose floral[g,h]
37	2-Methyl-1-butanol	HA_10	1214	1208 ± 95	x	x	x	x	MS, LRI	nail polish, malt[g]
38	3-Methyl-1-butanol	HA_11	1216	1209 ± 9	/	x	x	x	MS, LRI	nail polish, alcohol[f,g]
39	2-Methyl-1-propanol	HA_12	1104	1092 ± 9	/	x	x	x	MS, LRI	alcohol, nail polish, fusel, malt[f,g]
40	3-Methyl-1-pentanol	HA_13	1334	1326 ± 9	/	x	/	/	MS, LRI	pungent, alcohol, green[g]
Acids										
41	Acetic acid	A_1	1445	1449 ± 13	/	x	x	x	MS, LRI	volatile acidit, vinegar, sour[f,h,l]
42	2-Methylpropanoic acid	A_2	1576	1570 ± 13	/	x	x	x	MS, LRI	rancid, butter, cheese[f,l]
43	Hexanoic acid	A_3	1860	1846 ± 12	x	x	x	x	MS, LRI, STD	cheese, sweat[f,h,l]
44	Octanoic acid	A_4	2077	2060 ± 15	x	x	x	x	MS, LRI	rancid, fatty[l]
45	Decanoic acid	A_5	2285	2276 ± 15	/	x / /	x	x	MS, LRI	rancid fat[h]
46	3-Methylbutanoic acid	A_6	1677	1666 ± 11	/	/	x	x	MS, LRI	sweat, acid, rancid[k]
Aldehydes										
47	Hexanal	Ad_1	1082	1083 ± 8	x	/	/	/	MS, LRI, STD	grassy, fatty, herbaceous, green[f,g,h]
48	(2E)-2-hexenal	Ad_2	1223	1216 ± 9	x	/	/	/	MS, LRI	green, grassy, pungent[f,g]
49	Octanal	Ad_3	1292	1289 ± 9	x	x	/	x	MS, LRI, STD	green fat[h,j]
50	Nonanal	Ad_4	1397	1391 ± 8	x	x	/	/	MS, LRI, STD	green, slightly pungent[g]
51	Benzaldehyde	Ad_5	1535	1520 ± 14	/	/	x	x	MS, LRI, STD	roasted, almond, cherry[f,g]
52	Dodecanal	Ad_6	1718	1711 ± 11	/	x	/	x	MS, LRI	lily, fat, citrus[k]
Terpenoids										
53	3,7-Dimethyl-1,6-octadien-3-ol	T_1	1551	1547 ± 7	x	/	/	/	MS, LRI	citrus, floral, sweet, grape-like[g,l]
54	6-Methylhept-5-en-2-ol	T_2	1468	1465 ± 6	x	x	/	/	MS, LRI	rose[f]
55	α-Farnesene	T_3	1753	1745 ± 10	x	x	/	x	MS, LRI	wood sweet[h,k]
56	β-Damascenone	T_4	1840	1823 ± 14	x	x	x	x	MS, LRI, STD	apple, rose, honey[h,j]
Ketones										
57	3-Hydroxy-2-butanone	K_1	1296	1285 ± 12	/	x x /	x	x	MS, LRI	fatty, buttery, cream[f,g,l]
58	4-Methyl-2-pentanone	K_2	1010	1010 ± 7	x	/	x	x	MS, LRI	sulfur, strawberry, sweet, varnish[f,g]
59	2,6,8-Trimethyl-4-nonanone	K_3	1402	/	x	x	x	x	MS, LRI	/
60	6-Methylhept-5-en-2-one	K_4	1343	1339 ± 9	x	x	/	x	MS, LRI, STD	herbaceous, green, cabbage[g]
Lactones										
61	Dihydro-2(3H)-furanone	L_1	1648	1632 ± 15	/	/ x /	/	/ x /	MS, LRI	caramel sweet[h]
Acetals										
62	1-(1-Ethoxyethoxy) pentane	Ace_1	1096	1098 ± 8	/	x x /	/	/	MS, LRI	/
Sulfur										
63	3-(Methylsulfanyl)-1-propanol	S_1	1733	1719 ± 9	/	x	/	x / x	MS, LRI	sweet, potato[k]

ABS Alcoholic beverage sample; SC: *S. cerevisiae*; LT + SC: *L. thermotolerans* + *S. cerevisiae*, TD: *T. delbrueckii*.

[a] Number of volatiles investigated by DB-WAX.

[b] Kovats retention indices calculated using n-clcanes C7-C21.
[c] Retention indices from the National Institute of Standards and Technology database library from the software Chromeleon. Capillary column with DB-WAX active phase.
[d] Identification, MS: mass spectrum; LRI: literature retention index.
[e] Odor descriptors based on literature.
[f] He et al., 2021.
[g] Liu et al., 2019.
[h] Madrera et al., 2015.
[i] http://www.vcf-online.nl/VcfCompounds.cfm.
[j] Laaksonen et al., 2021.
[k] https://www.flavornet.org/flavornet.html.
[l] Liu Shuxun (2020) Doctoral thesis.

Table 3
Relative concentrations of the major volatile compounds (mg/L) identified in apple pomace mash, alcoholic beverage and vinegar samples.

Samples	Mash	Alcoholic beverage samples			Vinegar samples		
		SC	LT + SC	TD	SC	LT + SC	TD
Ethyl esters	0.62 ± 0.77 a	2029.89 ± 11.88 d	2982.40 ± 135.96 cd	1561.63 ± 41.78 e	1002.42 ± 298.31 b	1809.31 ± 550.17 bcd	1387.45 ± 401.77 bc
Acetate esters	41.41 ± 1.00 b	99.10 ± 2.84 b	236.54 ± 4.45 b	72.58 ± 9.48 b	307.29 ± 71.82 b	674.28 ± 237.38 a	646.73 ± 263.33 a
Other esters	167.23 ± 72.63 a	54.94 ± 5.86 bc	36.85 ± 1.06 c	63.05 ± 1.10 b	73.42 ± 6.65 b	63.71 ± 5.18 b	77.99 ± 4.46 b
Higher alcohols	327.77 ± 17.24 d	7347.97 ± 36.88 ab	6979.61 ± 294.19 b	7858.10 ± 387.63 a	1864.17 ± 406.88 c	1538.19 ± 434.03 c	1741.80 ± 180.36 c
Volatile acids	6.49 ± 0.38 c	418.75 ± 26.72 c	343.77 ± 14.83 c	381.90 ± 17.43 c	9572.01 ± 519.01 b	11519.10 ± 51.04 a	12023.74 ± 1070.1 a
Aldehydes	95.31 ± 5.61 a	5.66 ± 0.36 c	5.83 ± 1.33 bc	4.90 ± 1.06 c	14.80 ± 7.08 b	6.53 ± 0.00 bc	7.12 ± 0.77 bc
Terpenoids	293.57 ± 213.90 a	106.14 ± 3.66 b	77.30 ± 11.19 bc	90.43 ± 6.46 b	21.43 ± 3.91 c	21.73 ± 1.45 c	26.23 ± 4.26 c
Ketones	17.21 ± 1.25 d	35.88 ± 1.73 cd	28.14 ± 1.33 d	20.20 ± 1.03 cd	98.60 ± 2.53 b	209.10 ± 4.52 a	56.85 ± 19.86 c

Results represent the mean ± standard deviation. Mash samples in triplicate, alcoholic beverage samples in duplicate and vinegar samples in six replicates (2 biological replicates × 3 analytical replicates). Statistically significant differences between yeasts within each sample type, alcoholic beverage and vinegar, are shown with lower case letters a-e (ANOVA with Tukey's test). SC: *S. cerevisiae*; LT + SC: *L. thermotolerans* + *S. cerevisiae*, TD: *T. delbrueckii*.

To better show this separation of yeast in vinegar samples, another PCA was composed of 6 vinegar samples (biological duplicates of each yeast) and 63 volatile compounds (Fig. 2D). In this PCA, PC-1 explained 40.7% of the variance, separating SC samples from the rest of the samples. PC-2 explained 26.3% of the variance, with TD samples negatively correlated with LT + SC samples, indicating difference between these samples. SC vinegar samples were positively correlating to 1-hexanol (HA_3), 2-phenylethanol (HA_9), octanoic acid (A_4), decanoic acid (A_5), and 6-methylhept-5-en-2-one (K_4). TD vinegar samples were positively correlating to 2-methylpropanoic acid (A_2), 3-methylbutanoic acid (A_6), and α-farnesene (T_3). Finally, LT + SC vinegar samples were positively correlating to ethyl acetate (E_5), ethyl 2-hydroxypropanoate (E_6), butyl acetate (AE_2) and dihydro-2(3H)-furanone (L_1). This diversity confirmed that the selection of yeast strains plays an important role in determining the aroma of vinegar products.

4. Conclusion

This study characterized the effects of *Saccharomyces* and non-*Saccharomyces* strains on the chemical compositions of alcoholic beverage and vinegar products made from pomace of a Finnish apple cultivar. Yeast fermentation processes led to significant increases in the levels of volatile compounds, especially ethyl esters and higher alcohols in alcoholic beverages, whereas acetate esters, volatile acids, and ketones in vinegars. Alcoholic beverages fermented with *T. delbrueckii* had lower ethanol content compared to those fermented with *S. cerevisiae*, suggesting the potential use of non-*Saccharomyces* strains for producing alcoholic beverages with low ethanol content. Vinegars fermented with *L. thermotolerans* (sequential inoculation with *S. cerevisiae*) had the highest contents of total organic acids, ethyl esters and volatile acids, which may enhance the attributes of vinegar products.

Our study shows the potential utilization of non-*Saccharomyces* strains in vinegar production with the side streams from apple juice processing, thus valorizing apple pomace emerges as a promising and sustainable practice to increase the value of agricultural by-products. For future studies, the addition of a pure culture of acetic acid bacteria during the acetic fermentation process could be explored to compare with the addition of unpasteurized commercial vinegar (mother vinegar). The effect of these fermentations on the sensory quality of alcoholic beverages and vinegar products should also be further assessed using sensory evaluation. In addition, GC-O analysis and Odor Active Value (OAV) analysis could help understand better contribution of specific volatile compounds to the overall aroma profile.

CRediT authorship contribution statement

Qizai Wang: Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Oskar Laaksonen:** Writing – review & editing, Visualization, Supervision, Methodology, Funding acquisition, Data curation. **Elsa Xifre Pujol:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Maarit Heinonen:** Writing – review & editing, Resources. **Baoru Yang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Niina Kelanne:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation, Conceptualization.

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.

Acknowledgements

The study was financially supported by the Finland-China Food and

Health Network (award number: 26004241) and the Niemi Foundation (award number: 20230040). The authors thank Kaisa Pirilä for her contribution in the analysis of the sugars and organic acids.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.105447.

Data availability

Data will be made available on request.

References

Aprea, E., Charles, M., Endrizzi, I., Laura Corollaro, M., Betta, E., Biasioli, F., & Gasperi, F. (2017). Sweet taste in apple: The role of sorbitol, individual sugars, organic acids and volatile compounds. *Scientific Reports, 7*. https://doi.org/10.1038/srep44950

Asif, M., Javaid, T., Razzaq, Z., Khan, M., Maan, A., Yousaf, S., Usman, A., & Shahid, S. (2024). Sustainable utilization of apple pomace and its emerging potential for development of functional foods. *Environmental Science and Pollution Research, 31*(12). https://doi.org/10.1007/s11356-023-28479-9

Balázs, A., Tóth, M., Blazics, B., Héthelyi, É., Szarka, S., Ficsor, E., Ficzek, G., Lemberkovics, É., & Blázovics, A. (2012). Investigation of dietary important components in selected red fleshed apples by GC-MS and LC-MS. *Fitoterapia, 83*(8). https://doi.org/10.1016/j.fitote.2012.04.017

Benito, S. (2018a). The impact of *Torulaspora delbrueckii* yeast in winemaking. *Applied Microbiology and Biotechnology, 102*(7). https://doi.org/10.1007/s00253-018-8849-0

Benito, S. (2018b). The impacts of *Lachancea thermotolerans* yeast strains on winemaking. *Applied Microbiology and Biotechnology, 102*(Issue 16). https://doi.org/10.1007/s00253-018-9117-z

Budak, H. N. (2021). Alteration of antioxidant activity and total phenolic content during the eight-week fermentation of apple cider vinegar. *Horticultural Studies, 38*(1). https://doi.org/10.16882/hortis.882469

Chen, H., Chen, T., Giudici, P., & Chen, F. (2016). Vinegar functions on health: Constituents, sources, and formation mechanisms. *Comprehensive Reviews in Food Science and Food Safety, 15*(6). https://doi.org/10.1111/1541-4337.12228

Delgado, P., Sanz, M. T., Beltrán, S., & Núñez, L. A. (2010). Ethyl lactate production via esterification of lactic acid with ethanol combined with pervaporation. *Chemical Engineering Journal, 165*(2). https://doi.org/10.1016/j.cej.2010.10.009

Desiderio Estela Escalante, W. (2019). Perspectives and uses of non-*Saccharomyces* yeasts in fermented beverages. In *Frontiers and new trends in the science of fermented food and beverages*. https://doi.org/10.5772/intechopen.81868

Fan, W., Xu, Y., & Han, Y. (2011). Quantification of volatile compounds in Chinese ciders by stir bar sorptive extraction (SBSE) and gas chromatography-mass spectrometry (GC-MS). *Journal of the Institute of Brewing, 117*(1). https://doi.org/10.1002/j.2050-0416.2011.tb00444.x

FAOSTAT. (2023). *Crops and livestock products*. Rome: Food and Agriculture Organization of the United Nations. Available online: https://www.fao.org/faostat/en/#data/QCL. June 2024.

Fernandes, T., Silva-Sousa, F., Pereira, F., Rito, T., Soares, P., Franco-Duarte, R., & Sousa, M. J. (2021). Biotechnological importance of *Torulaspora delbrueckii*: From the obscurity to the spotlight. *Journal of Fungi, 7*(9). https://doi.org/10.3390/jof7090712

Ferreira, L., Perestrelo, R., Caldeira, M., & Câmara, J. S. (2009). Characterization of volatile substances in apples from Rosaceae family by headspace solid-phase microextraction followed by GC-qMS. *Journal of Separation Science, 32*(11). https://doi.org/10.1002/jssc.200900024

Fraternale, D., Ricci, D., Flamini, G., & Giomaro, G. (2011). Volatiles profile of red apple from marche region (Italy). *Records of Natural Products, 5*(3).

Guiné, R. P. F., Barroca, M. J., Coldea, T. E., Bartkiene, E., & Anjos, O. (2021). Apple fermented products: An overview of technology, properties and health effects. *Processes, 9*(2). https://doi.org/10.3390/pr9020223

He, W. (2022). *Bioprocessing of alcoholic beverages from apples and pears: Effects of raw materials and processes on quality*. University of Turku (in English). Doctoral thesis.

He, W., Laaksonen, O., Tian, Y., Heinonen, M., Bitz, L., & Yang, B. (2022). Phenolic compound profiles in Finnish apple (*Malus* × *domestica* Borkh.) juices and ciders fermented with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains. *Food Chemistry, 373*. https://doi.org/10.1016/j.foodchem.2021.131437

He, W., Liu, S., Heponiemi, P., Heinonen, M., Marsol-Vall, A., Ma, X., Yang, B., & Laaksonen, O. (2021). Effect of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* strains on chemical composition and sensory quality of ciders made from Finnish apple cultivars. *Food Chemistry, 345*. https://doi.org/10.1016/j.foodchem.2020.128833

Ho, C. W., Lazim, A. M., Fazry, S., Zaki, U. K. H. H., & Lim, S. J. (2017). Varieties, production, composition and health benefits of vinegars: A review. *Food Chemistry, 221*. https://doi.org/10.1016/j.foodchem.2016.10.128

Jo, D., Kim, G. R., Yeo, S. H., Jeong, Y. J., Noh, B. S., & Kwon, J. H. (2013). Analysis of aroma compounds of commercial cider vinegars with different acidities using SPME/GC-MS, electronic nose, and sensory evaluation. *Food Science and Biotechnology, 22*(6). https://doi.org/10.1007/s10068-013-0251-1

Johnston, C. S., White, A. M., & Kent, S. M. (2009). Preliminary evidence that regular vinegar ingestion favorably influences hemoglobin A1c values in individuals with type 2 diabetes mellitus. *Diabetes Research and Clinical Practice, 84*(2). https://doi.org/10.1016/j.diabres.2009.02.005

Kalaba, V., Marjanović Balaban, Ž., & Kalaba, D. (2019). Antibacterial activity of domestic apple cider vinegar. *AGRO SUR, 4*(1). https://doi.org/10.7251/agreng1901024k

Kalemba-Drożdż, M., Kwiecień, I., Szewczyk, A., Cierniak, A., & Grzywacz-Kisielewska, A. (2020). Fermented vinegars from apple peels, raspberries, rosehips, lavender, mint, and rose petals: The composition, antioxidant power, and genoprotective abilities in comparison to acetic macerates, decoctions, and tinctures. *Antioxidants, 9*(11). https://doi.org/10.3390/antiox9111121

Kelanne, N. (2021). *Novel bioprocessing for increasing consumption of Nordic berries*. University of Turku (in English). Doctoral thesis.

Kelanne, N., Laaksonen, O., Seppälä, T., Yang, W., Tuukkanen, K., Loponen, J., & Yang, B. (2019). Impact of cyclodextrin treatment on composition and sensory properties of lingonberry (*Vaccinium vitis-idaea*) juice. *Lebensmittel-Wissenschaft und -Technologie, 113*. https://doi.org/10.1016/j.lwt.2019.108295

Kelanne, N., Siegmund, B., Metz, T., Yang, B., & Laaksonen, O. (2022). Comparison of volatile compounds and sensory profiles of alcoholic black currant (*Ribes nigrum*) beverages produced with *Saccharomyces, Torulaspora*, and *Metschnikowia* yeasts. *Food Chemistry, 370*. https://doi.org/10.1016/j.foodchem.2021.131049

Laaksonen, O., Kahala, M., Marsol-Vall, A., Blasco, L., Järvenpää, E., Rosenvald, S., Virtanen, M., Tarvainen, M., & Yang, B. (2021). Impact of lactic acid fermentation on sensory and chemical quality of dairy analogues prepared from lupine (*Lupinus angustifolius L.*) seeds. *Food Chemistry, 346*. https://doi.org/10.1016/j.foodchem.2020.128852

Laaksonen, O., Kuldjärv, R., Paalme, T., Virkki, M., & Yang, B. (2017). Impact of apple cultivar, ripening stage, fermentation type and yeast strain on phenolic composition of apple ciders. *Food Chemistry, 233*. https://doi.org/10.1016/j.foodchem.2017.04.067

Lee, H. S., & Wrolstad, R. E. (1988). Apple juice composition: Sugar, nonvolatile acid, and phenolic profiles. *Journal - Association of Official Analytical Chemists, 71*(4). https://doi.org/10.1093/jaoac/71.4.789

Liu, S. (2020). *Fermentation with non-Saccharomyces yeasts as a novel biotechnology for berry wine production*. University of Turku (in English). Doctoral thesis.

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*. https://doi.org/10.1016/j.foodchem.2018.06.003

Liu, S., Laaksonen, O., Marsol-Vall, A., Zhu, B., & Yang, B. (2020). Comparison of volatile composition between alcoholic bilberry beverages fermented with non-*Saccharomyces* yeasts and dynamic changes in volatile compounds during fermentation. *Journal of Agricultural and Food Chemistry, 68*(11). https://doi.org/10.1021/acs.jafc.0c01050

Liu, S., Laaksonen, O., & Yang, B. (2019). Volatile composition of bilberry wines fermented with non-*Saccharomyces* and *Saccharomyces* yeasts in pure, sequential and simultaneous inoculations. *Food Microbiology, 80*. https://doi.org/10.1016/j.fm.2018.12.015

Lorenzini, M., Simonato, B., Slaghenaufi, D., Ugliano, M., & Zapparoli, G. (2019). Assessment of yeasts for apple juice fermentation and production of cider volatile compounds. *Lebensmittel-Wissenschaft und -Technologie, 99*. https://doi.org/10.1016/j.lwt.2018.09.075

Luzón-Quintana, L. M., Castro, R., & Durán-Guerrero, E. (2021). Biotechnological processes in fruit vinegar production. *Foods, 10*(Issue 5). https://doi.org/10.3390/foods10050945

Madrera, R. R., Bedriñana, R. P., & Valles, B. S. (2015). Production and characterization of aroma compounds from apple pomace by solid-state fermentation with selected yeasts. *Lebensmittel-Wissenschaft und -Technologie, 64*(2). https://doi.org/10.1016/j.lwt.2015.07.056

Mas, A., Torija, M. J., García-Parrilla, M. D. C., & Troncoso, A. M. (2014). Acetic acid bacteria and the production and quality of wine vinegar. *The Scientific World Journal, 2014*. https://doi.org/10.1155/2014/394671

Morata, A., Escott, C., Bañuelos, M. A., Loira, I., Del Fresno, J. M., González, C., & Suárez-lepe, J. A. (2020). Contribution of non-*Saccharomyces* yeasts to wine freshness. A review. *Biomolecules, 10*(Issue 1). https://doi.org/10.3390/biom10010034

Mosciano, G., Fasano, M., Cassidy, J., Connelly, K., Mazeiko, P., Montenegro, A., Allen, B., & Michalski, J. (1993). Organoleptic characteristics of flavor materials. *Perfumer & Flavorist, 18*, 43–45.

Mounir, M., Fauconnier, M.-L., Afechtal, M., Thonart, P., Alaoui, M. I., & Delvigne, F. (2018). Aroma profile of pilot plant-scale produced fruit vinegar using a thermotolerant *Acetobacter pasteurianus* strain isolated from Moroccan cactus. *Acetic Acid Bacteria, 7*(1). https://doi.org/10.4081/aab.2018.7312

O'Shea, N., Ktenioudaki, A., Smyth, T. P., McLoughlin, P., Doran, L., Auty, M. A. E., Arendt, E., & Gallagher, E. (2015). Physicochemical assessment of two fruit by-products as functional ingredients: Apple and orange pomace. *Journal of Food Engineering, 153*. https://doi.org/10.1016/j.jfoodeng.2014.12.014

Parapouli, M., Vasileiadis, A., Afendra, A. S., & Hatziloukas, E. (2020). *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiology, 6*(Issue 1). https://doi.org/10.3934/microbiol.2020001

Perestrelo, R., Silva, C. L., Silva, P., Medina, S., Pereira, R., & Câmara, J. S. (2019). Untargeted fingerprinting of cider volatiles from different geographical regions by

HS-SPME/GC-MS. *Microchemical Journal, 148*. https://doi.org/10.1016/j.microc.2019.05.028

Perussello, C. A., Zhang, Z., Marzocchella, A., & Tiwari, B. K. (2017). Valorization of apple pomace by extraction of valuable compounds. *Comprehensive Reviews in Food Science and Food Safety, 16*(5). https://doi.org/10.1111/1541-4337.12290

Porter, T. J., Divol, B., & Setati, M. E. (2019). *Lachancea* yeast species: Origin, biochemical characteristics and oenological significance. *Food Research International, 119*. https://doi.org/10.1016/j.foodres.2019.02.003

Qin, Z., Petersen, M. A., & Bredie, W. L. P. (2018). Flavor profiling of apple ciders from the UK and Scandinavian region. *Food Research International, 105*. https://doi.org/10.1016/j.foodres.2017.12.003

Shalini, R., & Gupta, D. K. (2010). Utilization of pomace from apple processing industries: A review. *Journal of Food Science and Technology, 47*(4). https://doi.org/10.1007/s13197-010-0061-x

Tocci, N., Egger, M., Hoellrigl, P., Sanoll, C., Beisert, B., Brezina, S., Fritsch, S., Schnell, S., Rauhut, D., & Conterno, L. (2023). *Torulaspora delbrueckii* strain behaviour within different refermentation strategies for sparkling cider production. *Applied Sciences, 13*(6). https://doi.org/10.3390/app13064015

Vicente, J., Kelanne, N., Navascués, E., Calderón, F., Santos, A., Marquina, D., Yang, B., & Benito, S. (2023). Combined use of *Schizosaccharomyces pombe* and a *Lachancea thermotolerans* strain with a high malic acid consumption ability for wine production. *Fermentation, 9*(2). https://doi.org/10.3390/fermentation9020165

Vicente, J., Navascués, E., Calderón, F., Santos, A., Marquina, D., & Benito, S. (2021). An integrative view of the role of *Lachancea thermotolerans* in wine technology. *Foods, 10*(Issue 11). https://doi.org/10.3390/foods10112878

Vilela, A. (2018). *Lachancea thermotolerans*, the Non-*Saccharomyces* yeast that reduces the volatile acidity of wines. *Fermentation, 4*(Issue 3). https://doi.org/10.3390/fermentation4030056

Villire, A., Arvisenet, G., Lethuaut, L., Prost, C., & Sérot, T. (2012). Selection of a representative extraction method for the analysis of odourant volatile composition of French cider by GC-MS-O and GC × GC-TOF-MS. *Food Chemistry, 131*(4). https://doi.org/10.1016/j.foodchem.2011.10.008

Viroli, S. L. M., Viroli, S. G., Carvalho, N. P., Bernardi, D. P. da S., & Coelho, R. G. (2021). Production and characterization of acetic fermentation with different fruit peels. *Research, Society and Development, 10*(14). https://doi.org/10.33448/rsd-v10i14.21878

Wang, Y., Qi, X., Fu, Y., Zhang, Q., Wang, X., Cui, M., Ma, Y., & Gao, X. (2023). Effects of *Torulaspora delbrueckii* co-fermented with *Saccharomyces cerevisiae* on physicochemical and aromatic profiles of blueberry fermented beverage. *Food Chemistry, 409*. https://doi.org/10.1016/j.foodchem.2022.135284

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). *Understanding wine chemistry* (1st ed.). John Wiley & Sons, Ltd. Online, 9781118730720.

Wei, J., Zhang, Y., Wang, Y., Ju, H., Niu, C., Song, Z., Yuan, Y., & Yue, T. (2020). Assessment of chemical composition and sensorial properties of ciders fermented with different non-*Saccharomyces* yeasts in pure and mixed fermentations. *International Journal of Food Microbiology, 318*. https://doi.org/10.1016/j.ijfoodmicro.2019.108471

Xia, T., Zhang, B., Duan, W., Zhang, J., & Wang, M. (2020). Nutrients and bioactive components from vinegar: A fermented and functional food. *Journal of Functional Foods, 64*. https://doi.org/10.1016/j.jff.2019.103681

Xu, Y., Fan, W., & Qian, M. C. (2007). Characterization of aroma compounds in apple cider using solvent-assisted flavor evaporation and headspace solid-phase microextraction. *Journal of Agricultural and Food Chemistry, 55*(8). https://doi.org/10.1021/jf0631732

Yang, S., Hao, N., Meng, Z., Li, Y., & Zhao, Z. (2021). Identification, comparison and classification of volatile compounds in peels of 40 apple cultivars by HS–SPME with GC–MS. *Foods, 10*(5). https://doi.org/10.3390/foods10051051

Ye, M., Yue, T., & Yuan, Y. (2014). Changes in the profile of volatile compounds and amino acids during cider fermentation using dessert variety of apples. *European Food Research and Technology, 239*(1), 67–77. https://doi.org/10.1007/s00217-014-2204-1

Zhang, F., Wang, T., Wang, X., & Lü, X. (2021). Apple pomace as a potential valuable resource for full-components utilization: A review. *Journal of Cleaner Production, 329*. https://doi.org/10.1016/j.jclepro.2021.129676