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Effect of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* fermentation on chemical composition of ciders made from traditional Finnish apple cultivars

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ABSTRACT

This study investigated the impact of two yeast strains, *Saccharomyces cerevisiae* (SC) and *Torulaspora delbrueckii* (TD), on the chemical composition of ciders prepared from the juices of six traditional apple cultivars grown in Finland, aiming to evaluate the potential of these underutilized local cultivars for cider production. Sugars, organic acids and ethanol contents were analyzed using gas chromatography coupled with a flame ionization detector (GC–FID), while volatile compounds were identified and semi-quantified using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS–SPME–CG–MS). Apple cultivar selection had a stronger impact than yeast strain on the sugars and organic acids in ciders, whereas both factors shaped the volatile compound profiles. Among the cultivars, 'Rambo' demonstrated the greatest potential for cider aroma development. Specifically, fermentation of 'Rambo' juice with TD yielded the highest ester production among all TD-fermented ciders. Volatile profiling further revealed that TD favored the formation of fruity esters while reducing higher alcohol and acetic acid levels, resulting in potentially more complex and fruity aroma, compared to SC. Other cultivar–yeast combinations, such as 'Aleksanteri', 'Antonovka', or 'Mustialan Iso Venäläinen' with SC, could also enhance cider aroma complexity. These findings highlight the potential of underutilized apple cultivars, when combined with tailored yeast selection, to diversify cider profiles and to enhance sensory quality.

1. Introduction

Apple cider is a globally growing alcoholic beverage category, with Europe remaining the dominant consumption region (European Cider and Fruit Wine Association [AICV] & GlobalData, 2024). Notably, the chemical profile of cider is intrinsically linked to the geographic origin and cultivar of the apples used, as demonstrated by studies from diverse regions such as Madeira Island, the UK, and Scandinavian region (Qin et al., 2018; Sousa et al., 2020). This geographic specificity underpins the potential for developing distinctive ciders derived from region-specific apple genetics.

In Finland, apple cultivation faces challenges due to the harsh climate, but a range of traditional and hardy cultivars exist (Garkava-Gustavsson et al., 2013; Heinonen & Bitz, 2019). However, new varieties have replaced many traditional ones in apple cultivation because they are better suited for fresh consumption. In contrast, traditional varieties may prove suitable to produce a local specialty cider. Though interest in using these local cultivars for cider production is growing, there is a lack of targeted breeding programs and data on their cider-making potential, particularly regarding their basic chemical composition (He et al., 2021). Therefore, investigating underutilized locally grown traditional cultivars is essential to support the development of a regionally distinctive cider sector.

The aroma and quality of cider are primarily determined by its volatile organic compounds (VOC), which is influenced by a range of factors, including apple cultivar, ripeness, fermentation parameters and

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yeast strain (Medina et al., 2020; Zeng et al., 2025). Different yeasts possess distinct metabolic profiles leading to diverse chemical composition in the final product (Bingman et al., 2021; Li et al., 2026). While *Saccharomyces cerevisiae* (SC) is the conventional choice for cider fermentation (Al Daccache et al., 2020), non-*Saccharomyces* yeasts, such as *Torulaspora delbrueckii* (TD), are gaining attention for their distinct metabolic outcomes. In particular, TD has been shown to reduce acetic acid levels during wine fermentation and enhance the production of compounds associated with fruity aromas, such as esters and terpenes (Belda et al., 2017; Călugăr et al., 2024; Fernandes et al., 2021; Renault et al., 2016).

In this study, we investigated the cider-making potential of non-commercial, traditional apple cultivars from an old orchard in Turku, Finland. This orchard serves as a representative example of many similar old orchards in Finland that remain largely underutilized as a resource for cider production. As an initial assessment, we characterized how apple cultivar and yeast strain selection (SC versus TD) influence the chemical composition of the resulting ciders from a single harvest year. The aim was not only to evaluate these specific cultivars per se, but also to explore the broader potential of apples from old orchards as a poorly utilized raw material. Sugar, organic acid and ethanol contents were analyzed by gas chromatography coupled with flame ionization detector (GC–FID), and VOC profiles were characterized by headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS–SPME–GC–MS). The comparative analysis provides a basis for identifying promising apples from old orchards and similar underutilized resources for cider production.

2. Material and methods

2.1. Apple cultivars

Six traditional apple cultivars were used, including four identified cultivars: 'Antonovka' (AN), 'Aleksanteri' (AL), 'Rambo' (RA) and 'Mustialan Iso Venäläinen' (MV) and two unknown cultivars (T1, T2) (Table S1 and Fig. S1). Apples were grown in the historical garden of St Mary's Church, Turku, Southwest Finland (60°25′N, 22°31′E) and harvested in autumn 2022. Only visually intact apples without apparent defects were selected. Apples were stored at 4 °C for four weeks before juice processing.

2.2. Chemicals and standards

Ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl decanoate, ethyl dodecanoate, hexyl acetate, butyl butanoate, butyl hexanoate, hexyl butanoate, 1-octen-3-ol, 1-octanol, hexanoic acid, hexanal, 6-methylhept-5-en-2-one, β-damascenone, 4-methyl-2-pentanol, alkane mixture (C5–C30), tartaric acid, xylitol, quinic acid and malic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Butyl acetate, ethyl hexanoate and D-sorbitol were purchased from Fluka Chemicals (Neu-Ulm, Bavaria, Germany). Succinic acid, D-(+)-glucose and D-(−)-frucose were purchased from Merck (Darmstadt, Hesse, Germany). Citric acid and sucrose were purchased from J.T. Baker Chemicals (Leuven, Belgium). Ascorbic acid was purchased from Extrasynthese (Genay, France). Tri-Sil® HTP reagent was purchased from Thermo Scientific (Bellefonte, PA, USA). Ethanol (≥99.5%) was purchased from Altia Oy (Rajamäki, Uusimaa, Finland).

2.3. Methods

2.3.1. Apple juice preparation

Apples were washed, cored, and cut into pieces prior to juice extraction using a Kenwood Chef XL juice extractor with a mixer attachment AT641 (Kenwood Ltd., Havant, Hampshire, UK). All cultivars were processed at the same time. The resulting unfiltered juices (T1J, T2J, ANJ, ALJ, RAJ and MVJ) were stored at −22 °C for up to seven weeks prior to chemical analysis and yeast inoculation. Juices were not thermally treated and no additives were applied. An overview of the cider production workflow from apple sorting to cider sample collection is shown in Fig. 1.

2.3.2. Apple cider preparation

Active dry yeasts LALVIN PERSY™ (SC) and BIODIVA™ TD291 (TD) were obtained from Lallemand Biofuels & Distilled Spirits (Milwaukee, WI, USA). Yeast rehydration, inoculation and fermentation monitoring were performed following previously reported methodologies (He et al., 2021; Wang et al., 2024). Yeasts were rehydrated in the nutrient solution Fermaid K (Lallemand Inc, Montreal, QC, Canada) according to recommendations by the manufacturer and inoculated into freshly thawed apple juice. Final inoculation levels were approximately 4.9×10^9 CFU/mL for SC and 4.7×10^8 CFU/mL for TD. Bottles were agitated to ensure uniform yeast dispersion. Fermentations were conducted in triplicate at room temperature (22 °C) in the dark. Bottles were sealed with caps and opened every three days to release CO_2. Fermentation progress was monitored every three days by measuring °Brix values using a refractometer (Hanna Instruments, Woonsocket, RI, USA) and recording bottle weight. Fermentation was terminated by adding potassium sorbate and potassium sulphate (1:1, Jässtopp D, Viinitialo Melkko, Lahti, Finland) when °Brix and bottle weight remained constant for four consecutive days.

Samples were then stored overnight at 4 °C, centrifuged (Avanti JXN-26 centrifuge; Beckman Coulter, Indianapolis, IN, USA) at 8000×*g* for 10 min at 4 °C, and the supernatants were collected. The resulting ciders (T1S, T2S, ANS, ALS, RAS, MVS fermented with SC; and T1T, T2T, ANT, ALT, RAT, MVT fermented with TD) were stored at −22 °C for up to two weeks prior to chemical analysis. All fermented samples were analyzed in biological and analytical triplicates.

2.3.3. Analysis of ethanol contents

Ethanol concentration in cider samples was determined in triplicate by GC (Shimadzu Nexis GC-2030; Shimadzu Corp., Kyoto, Japan), equipped with an autoinjector (AOC-20i), an autosampler (AOC-20s), a flame ionization detector (FID), and an HP-INNOWax column (30 m × 0.25 mm i.d., 0.25 μm; Hewlett-Packard, Avondale, PA, USA), following the method of Wang et al. (2024). Quantification was performed using a five-point external standard curve (0%–10%, v/v).

2.3.4. Analysis of sugars and organic acids

Sugars and acids were analyzed as trimethylsilyl (TMS) derivatives using GC–FID (GC-2010Plus, Shimadzu Corp.), based on Wang et al. (2024) with minor modifications. Both internal (for quantification) and external (for identification and for correction factor calculations) standards were used. Internal standards were tartaric acid and xylitol in 5 g/L. External standards were applied for identification and quantification: quinic acid (5.388 g/L), malic acid (5.004 g/L), citric acid (7.268 g/L), succinic acid (2.392 g/L), sucrose (7.348 g/L), D-(+)-glucose (6.916 g/L), and D-(−)-frucose (4.904 g/L). Concentrations were calculated according to Equation (1):

$$C_{analyte}(g/L) = \frac{A_{analyte} \times C_{standard}}{k \times A_{standard}} \quad (1)$$

Where A is peak area, C is concentration, and k is the correction factor.

2.3.5. Analysis of volatile compounds

The VOC profile was analyzed by HS–SPME–GC–MS using 4-methyl-2-pentanol (802 μg/mL in methanol) as the internal standard, following Vicente et al. (2023). Samples (2 mL) were equilibrated with NaCl (0.2 g) in 20 mL vials and extracted using a 2 cm DVB/CAR/PDMS fiber (50/30 μm, Supelco, Bellefonte, PA, USA). Separation was achieved on a DB-WAX polar capillary column (60 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA, USA). The VOC was identified by comparison

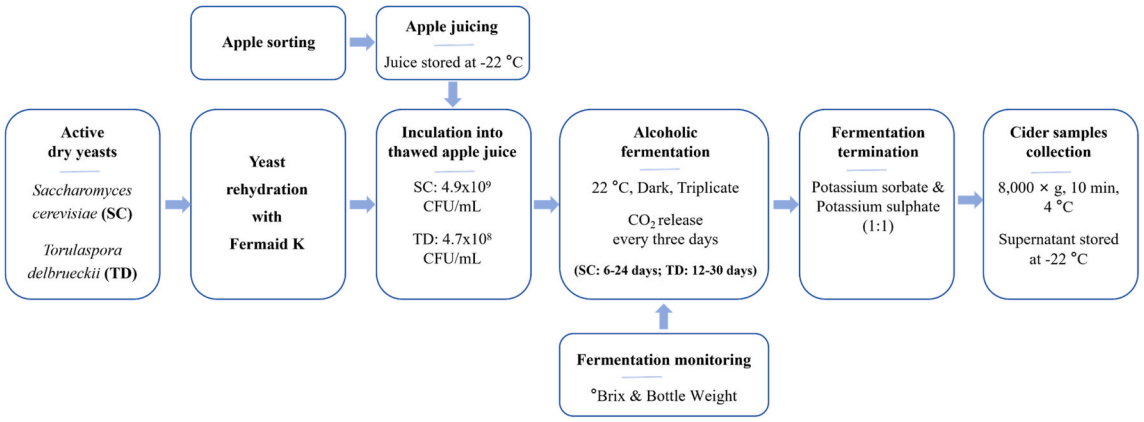

Fig. 1. Workflow of cider fermentation and sample preparation.

with the NIST 20 library and retention indices calculated using C5–C30 alkanes (He et al., 2021; Vicente et al., 2023). Semi-quantification was performed by normalizing their peak area to that of the internal standard.

2.3.6. Statistical analysis

Differences among apple cultivars and fermentations were evaluated using one-way Analysis of Variance (ANOVA), Tukey's test and t-tests in IBM SPSS Statistics 29.0 (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $p < 0.05$. Multivariate analyses, including Partial Least Squares Discriminate Analysis (PLS-DA) and Principal Components Analysis (PCA), were conducted using Unscrambler X (version 11.0; Camo Analytics as., Oslo, Norway).

3. Results and discussion

3.1. Juice processing

Juice yield varied among the apple cultivars, ranging from 40.73% (MV) to 51.99% (RA) (Table S1). T1, AN, and MV exhibited internal rot and less juicy flesh, resulting in lower yields and higher processing waste. In contrast, RA yielded the most juice despite the smallest processed batch. This variation in yield reflects the cultivar-specific physical properties of the fruit, which in turn define the initial substrate for the subsequent fermentation.

3.2. General fermentation parameters

Fermentation kinetics revealed a distinct physiological difference between yeast strains (Fig. 2). The SC strains completed fermentations within 6–24 days, whereas the TD strains required 12–30 days, consistent with its longer lag phase reported in previous studies (Kelanne et al., 2020; Wang et al., 2023). In all fermentations, total soluble solids (TSS) decreased consistently, with yeast activity diminishing only when TSS approached approximately 3.6–4.1 °Brix. Despite of the difference in fermentation rate, the final average ethanol content did not differ statistically between yeast stains (Table 1). Instead, ethanol production correlated more strongly with the initial TSS of the must. For example, RAJ exhibited the highest TSS (13.70 °Brix) and consequently produced the most alcoholic ciders (RAS: 7.81%; RAT: 7.65%). This is supported by similar findings of He et al. (2021) indicating that ethanol yield was more strongly influenced by the apple cultivar rather than the yeast strain.

3.3. Sugar and organic acids in apple juices and ciders

The changes in sugar and organic acid profiles following fermentation were calculated using Equation (1)and the results are presented in Table 1. Fermentation fundamentally reshaped the profile of sugars and organic acids, yet the resulting composition was predominantly defined by the apple cultivar rather than the yeast strain. Apple juices exhibited a wide range in initial sugar content, from 26.08 g/L (MVJ) to 73.33 g/L (RAJ). Approximately 80%–90% of total sugars (primarily sucrose and

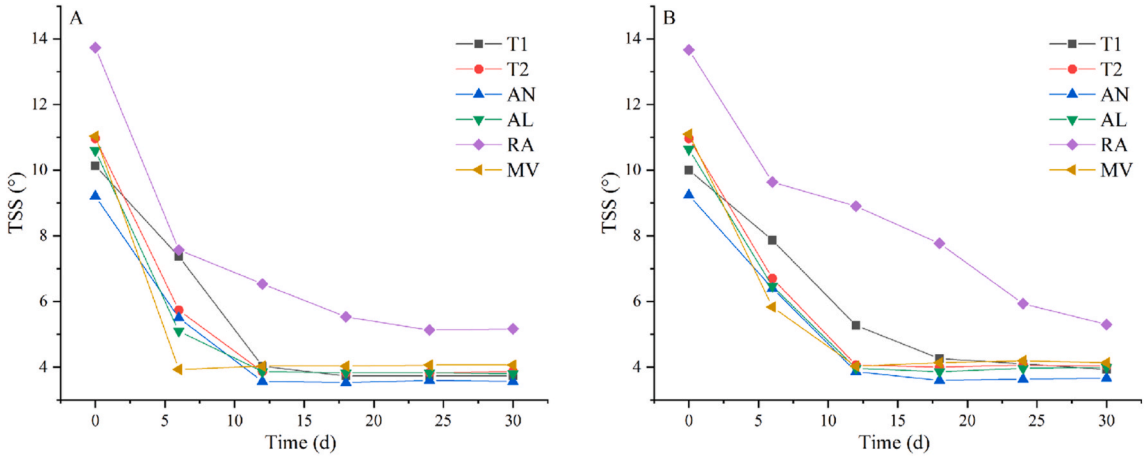

Fig. 2. Fermentation kinetics during alcoholic fermentations. TSS (total soluble solids) contents during fermentation process with A) *S. cerevisiae*; B) *T. delbrueckii*. Apple cultivars: 'Tunnistamaton1' (T1), 'Tunnistamaton2' (T2), 'Antonovka' (AN), 'Aleksanteri' (AL), 'Rambo' (RA) and 'Mustialan Iso Venäläinen' (MV).

Table 1
Chemical composition of juices from six cultivars and theirs corresponding ciders fermented with *S. cerevisiae* and *T. delbrueckii*.

Sample	TSS (°Brix)	Total Weight loss (g)	Ethanol content (v/v, %)	Sucrose (g/L)	Fructose (g/L)	Glucose (g/L)	Sorbitol (g/L)	Total sugars (g/L)	Malic acid (g/L)	Quinic acid (g/L)	Ascorbic acid (g/L)	Succinic acid (g/L)	Citric acid (g/L)	Total acids (g/L)	Sugar/acid ratio
Juices															
T1J	10.07 ± 0.12 c	/	/	8.86 ± 0.17 b	9.50 ± 0.20 b	6.38 ± 0.15 ab	2.86 ± 0.07 b	27.60 ± 0.58 b	10.17 ± 1.26 ab	0.56 ± 0.04 ab	0.50 ± 0.16	ND	0.11 ± 0.09 ab	11.34 ± 1.49 abc	2.46 ± 0.30 bc
T2J	10.97 ± 0.60 b	/	/	13.12 ± 3.55 b	9.61 ± 2.75 b	3.27 ± 0.83 b	2.21 ± 0.66 b	28.21 ± 7.78 b	9.20 ± 2.47 ab	0.58 ± 0.15 ab	0.44 ± 0.07	ND	0.11 ± 0.09 ab	10.33 ± 2.78 abc	2.74 ± 0.22 bc
ANJ	9.22 ± 0.04 d	/	/	7.98 ± 0.72 b	10.01 ± 1.14 a	6.15 ± 2.50 ab	2.46 ± 0.22 b	26.59 ± 3.71 b	13.45 ± 0.86 a	0.56 ± 0.04 ab	0.20 ± 0.18	ND	0.17 ± 0.09 ab	14.38 ± 0.98 ab	1.85 ± 0.24 c
ALJ	10.62 ± 0.29 b	/	/	10.29 ± 0.73 b	9.62 ± 0.54 b	4.69 ± 0.32 b	3.74 ± 0.21 b	28.34 ± 1.74 b	7.96 ± 0.62 ab	0.42 ± 0.25 b	ND	ND	ND	8.37 ± 0.79 bc	3.39 ± 0.11 ab
RAJ	13.70 ± 0.09 a	/	/	33.30 ± 9.47 a	16.12 ± 4.50 ab	10.49 ± 3.01 a	13.43 ± 3.86 a	73.33 ± 20.82 a	13.63 ± 3.96 a	0.99 ± 0.31 a	0.60 ± 0.59	ND	0.38 ± 0.25 a	15.59 ± 4.57 a	4.72 ± 0.32 a
MVJ	11.07 ± 0.16 b	/	/	9.09 ± 0.88 b	9.08 ± 1.33 b	4.82 ± 0.65 b	3.09 ± 1.17 b	26.08 ± 1.73 b	6.11 ± 2.10 b	0.61 ± 0.26 ab	0.38 ± 0.38	ND	0.08 ± 0.08 ab	7.18 ± 1.97 c	3.85 ± 1.22 ab
Mean	10.94 ± 1.43 A	/	/	13.77 ± 9.79	10.66 ± 3.17	5.97 ± 2.72	4.63 ± 4.31	35.03 ± 19.29 A	10.09 ± 3.37 A	0.62 ± 0.25 A	0.35 ± 0.33	/	0.14 ± 0.16	11.20 ± 3.72	3.17 ± 1.08 A
S.cerevisiae ciders															
T1S	3.73 ± 0.06 c	6.61 ± 0.14 cd	4.57 ± 1.52 b	ND	ND	ND	3.20 ± 0.37 bc	3.20 ± 0.37 bc	9.37 ± 0.57 ab	0.35 ± 0.11 bc	0.19 ± 0.21 c	1.06 ± 0.11	0.12 ± 0.02 b	11.10 ± 0.66 ab	0.29 ± 0.03 c
T2S	3.87 ± 0.23 bc	7.46 ± 0.31 bc	5.37 ± 1.19 b	ND	ND	ND	2.51 ± 0.46 c	2.51 ± 0.46 c	7.80 ± 0.84 bc	0.21 ± 0.03 c	0.30 ± 0.23 bc	0.87 ± 0.08	0.11 ± 0.02 bc	9.29 ± 0.86 b	0.27 ± 0.03 cd
ANS	3.57 ± 0.06 c	5.99 ± 0.36 d	4.18 ± 1.11 b	ND	ND	ND	2.24 ± 0.49 c	2.24 ± 0.49 c	10.47 ± 1.92 a	0.28 ± 0.10 c	0.39 ± 0.14 bc	0.99 ± 0.10	0.18 ± 0.05 a	12.32 ± 2.08 a	0.18 ± 0.02 d
ALS	3.80 ± 0.10 bc	6.91 ± 0.41 bcd	4.09 ± 1.55 b	ND	ND	ND	3.81 ± 0.28 bc	3.81 ± 0.28 bc	6.79 ± 0.84 c	0.46 ± 0.04 b	0.36 ± 0.27 bc	0.95 ± 0.08	0.08 ± 0.03 bcd	8.64 ± 1.05 bc	0.45 ± 0.04 b
RAS	5.17 ± 0.06 a	9.28 ± 0.39 a	7.81 ± 0.35 a	ND	ND	ND	8.65 ± 2.59 a	8.65 ± 2.59 a	8.19 ± 2.53 bc	0.63 ± 0.14 a	0.88 ± 0.35 a	1.07 ± 0.33	0.07 ± 0.04 cd	10.85 ± 3.29 ab	0.80 ± 0.10 a
MVS	4.07 ± 0.06 b	7.70 ± 0.35 b	4.64 ± 1.63 b	ND	ND	ND	4.83 ± 1.35 b	4.83 ± 1.35 b	4.22 ± 1.13 d	0.35 ± 0.16 bc	0.60 ± 0.21 ab	0.91 ± 0.23	0.06 ± 0.05 d	6.15 ± 1.67 c	0.79 ± 0.13 a
Mean	4.03 ± 0.55 B	7.32 ± 1.10	5.11 ± 1.79	/	/	/	4.21 ± 2.48	4.21 ± 2.48 B	7.81 ± 2.45 B	0.38 ± 0.17 B	0.45 ± 0.33	0.98 ± 0.19	0.11 ± 0.05	9.72 ± 2.67	0.46 ± 0.26 B
T.delbrueckii ciders															
T1T	3.93 ± 0.15 bc	6.29 ± 0.40 cd	4.75 ± 0.99 bc	ND	ND	ND	3.81 ± 0.70 cd	3.81 ± 0.70 cd	9.05 ± 1.57 b	0.46 ± 0.09 b	0.34 ± 0.30 b	1.91 ± 0.32 a	0.14 ± 0.04 b	11.89 ± 2.18 ab	0.32 ± 0.01 d
T2T	4.03 ± 0.29 bc	7.37 ± 0.34 b	5.48 ± 1.00 b	ND	ND	ND	2.58 ± 1.05 e	2.58 ± 1.05 e	9.30 ± 1.12 b	0.28 ± 0.08 c	0.33 ± 0.14 b	1.05 ± 0.12 c	0.13 ± 0.03 b	11.09 ± 1.28 b	0.23 ± 0.09 de
ANT	3.67 ± 0.06 c	5.78 ± 0.26 d	4.40 ± 0.20 bc	ND	ND	ND	2.93 ± 0.41 de	2.93 ± 0.41 de	11.46 ± 1.78 a	0.32 ± 0.07 c	0.31 ± 0.25 b	1.47 ± 0.21 b	0.20 ± 0.05 a	13.76 ± 1.75 a	0.22 ± 0.05 e
ALT	4.00 ± 0.20 bc	7.02 ± 0.20 bc	3.35 ± 1.26 c	ND	ND	ND	4.36 ± 0.73 bc	4.36 ± 0.73 bc	7.96 ± 1.39 b	0.49 ± 0.08 b	0.39 ± 0.35 b	1.44 ± 0.16 b	0.10 ± 0.03 bc	10.39 ± 1.36 b	0.43 ± 0.08 c
RAT	5.30 ± 0.00 a	8.77 ± 0.07 a	7.65 ± 0.42 a	ND	ND	ND	10.77 ± 0.52 a	10.77 ± 0.52 a	8.26 ± 0.32 b	0.61 ± 0.03 a	1.03 ± 0.18 a	1.88 ± 0.07 a	0.09 ± 0.01 bc	11.87 ± 0.56 ab	0.91 ± 0.04 a
MVT	4.13 ± 0.12 b	7.00 ± 0.30 bc	5.06 ± 1.74 b	ND	ND	ND	5.31 ± 1.25 b	5.31 ± 1.25 b	4.62 ± 1.20 c	0.39 ± 0.11 bc	0.55 ± 0.25 b	0.99 ± 0.23 c	0.06 ± 0.04 c	6.61 ± 1.78 c	0.81 ± 0.08 b
Mean	4.18 ± 0.55 B	7.04 ± 0.99	5.11 ± 1.67	/	/	/	4.96 ± 2.89	4.96 ± 2.89 B	8.44 ± 2.41 B	0.43 ± 0.14 B	0.49 ± 0.35	1.46 ± 0.41	0.12 ± 0.06	10.94 ± 2.67	0.49 ± 0.28 B

Results represent the mean ± standard deviation. Juice samples in triplicate, cider samples in nine replicates (3 biological replicates × 3 analytical replicates). ND: not detected. Statistically significant differences between cultivarss with each sample type are shown with lower case letter a-e and for mean values are shown with upper case letters A-B (One-way ANOVA test, Tukey's test and T-test; $p < 0.05$). Apple cultivars: 'Tunnistamaton1' (T1), 'Tunnistamaton2' (T2), 'Antonovka' (AN), 'Aleksanteri' (AL), 'Rambo' (RA) and 'Mustialan Iso Venäläinen' (MV). The last letter of sample name: J means Juice; S means cider fermented with *S. cerevisiae;* T means cider fermented with *T. delbrueckii*.

fructose) were consumed by following fermentation, leaving sorbitol as the only residual sugar in all ciders. Its final concentration varied significantly ($p < 0.05$) from 2.24 g/L (ANS) to 10.77 g/L (RAT), and was consistently higher in ciders fermented with TD. This may be attributed to the reported ability of TD to produce significant amounts of C5 and C6 polyols, including D-sorbitol, during alcoholic fermentation (Mbuyane et al., 2018). The acid profile was similarly transformed. Malic acid was the dominant organic acid in both juices and ciders, with RAJ showing the highest level (13.63 g/L), accounting for 87.40% of its total acidity. High malic acid concentration may enhance perceived acidity and astringency (Castro et al., 2025; Payan et al., 2023). Succinic acid appeared only after fermentation and reached its highest concentration in T1T (1.91 g/L), almost twice that observed in SC-fermented cider (RAS 1.07 g/L). This confirms a strain-specific metabolic difference, an observation aligns with Fernandes et al. (2021), who reported higher succinic acid production by TD compared to SC and noted that TD maintains respiratory metabolism for longer under oxygen-limited conditions, which may contribute to its higher succinic acid production during fermentation. As these concentrations exceed the reported sensory threshold (0.68 g/L; Kelanne et al., 2020), the level of succinic acid detected here likely contributed to the sour and bitter perception in all ciders. Furthermore, fermentation markedly reduced the sugar-to-acid ratio in all samples (Table 1). RAT maintained the highest value (0.91) and ANS the lowest (0.18), indicating that cultivar selection strongly affects the perceived sweetness-acidity balance.

Two PCA plots were performed to illustrate differences in sugars and organic acids between juices (n = 6) and ciders (n = 18) fermented with SC or TD (Fig. 3 A&B). The clear separation of juices from ciders along PC-1 (explaining 51% of the total variance) quantified the profound impact of fermentation (Fig. 3A). More importantly, PCA of the ciders alone revealed that samples clustered primarily by apple cultivar, not by yeast strain (Fig. 3B), indicating that the cultivar exerted a stronger influence than the yeast on the final sugar and organic acid composition.

3.4. Volatile compounds in apple juices and ciders

3.4.1. Volatile composition of juices and ciders

A total of 64 VOC were identified across juice and their corresponding cider samples (Table 2). Detailed semi-quantitative data for the identified volatile compounds are provided in Table S2, with the aggregated content of each volatile compound class reported in Table 3. Fermentation substantially expanded the volatile profile: 28 compounds, mainly ethyl esters, higher alcohols, and volatile acids, appeared exclusively in ciders, whereas 15 compounds, primarily other esters present in the juice, were no longer detectable, increasing the total VOC diversity from 36 in juices to 49 in ciders.

Esters, which contribute to fruity aromas in fermented beverages (Zeng et al., 2025), were among the major groups of VOC in both juices

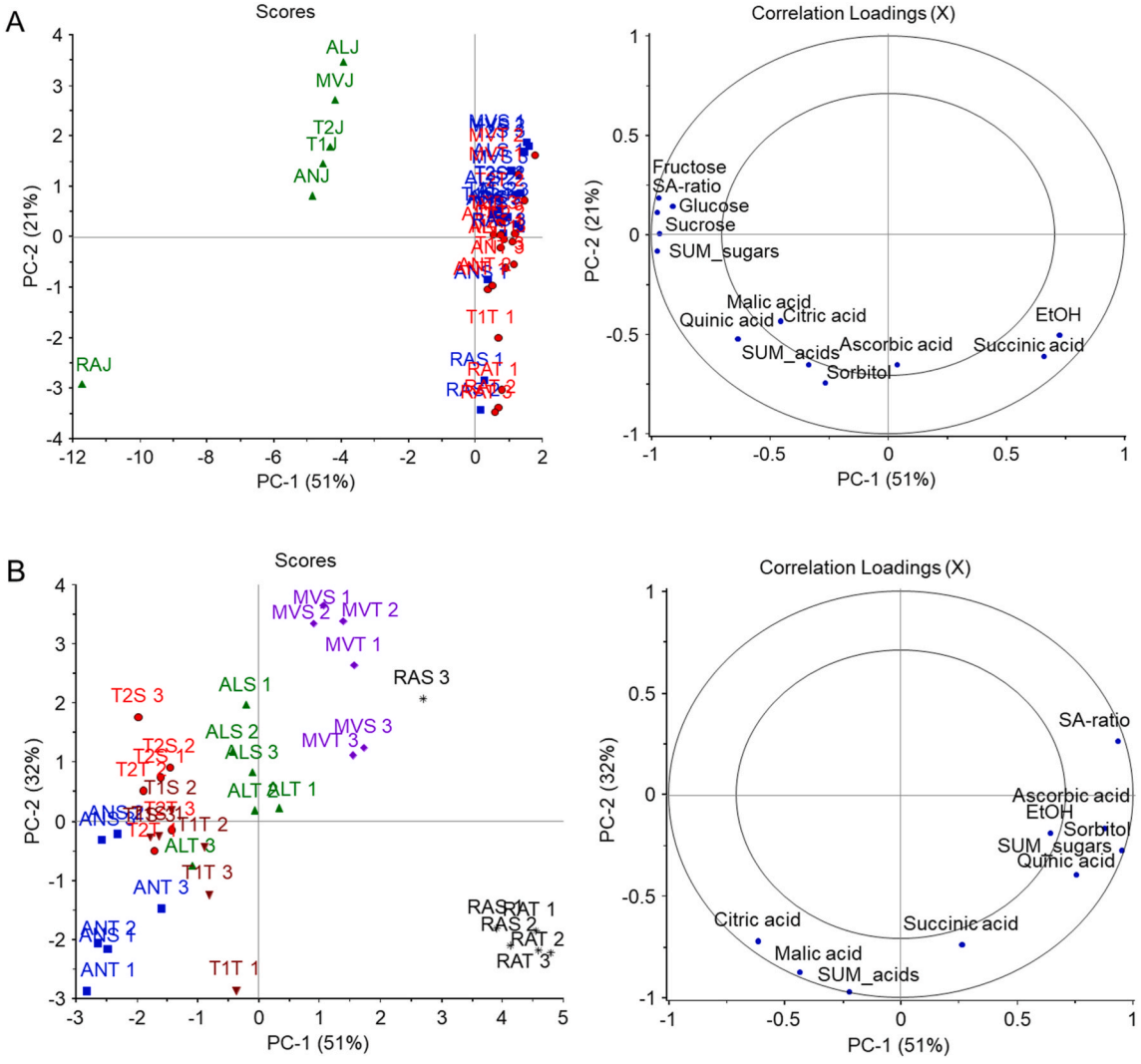

Fig. 3. Principal Component Analysis (PCA) of sugars and acid variables in the samples (J juices, S *S. cerevisiae* ciders, T *T. delbrueckii* ciders). A. Juices (n = 6) and ciders (n = 18), B. only cider samples (n = 18). Abbreviations refer to Table 1. Data is averaged from triplicate analyses to biological replicates (shown with 1-3).

Table 2
Identification of volatile compounds using HS-SPME-GC-MS in apple juice and cider samples fermented with *S. cerevisiae* and *T. delbrueckii*.

Peak number[a]	Compounds	RI		Presence		Identification[c]	Odor descriptor[d]
		Measured[b]	Literature	Juice	Cider		
Ethyl esters							
1	Ethyl Acetate	888	888	×	×	MS, LRI	pineapple, sweet, pungent[e]
2	Ethyl butanoate	1037	1036	×	×	MS, LRI, STD	fruity[e]
3	Ethyl 2-methylbutanoate	1052	1052	×	×	MS, LRI, STD	fruity[e]
4	Ethyl 3-hydroxybutanoate	1522	1515	×	×	MS, LRI	grape, roasted nut[e]
5	Ethyl 3-methylbutanoate	1068	1068		×	MS, LRI, STD	fruity, apple[f]
6	Ethyl hexanoate	1234	1233		×	MS, LRI, STD	green apple, brandy, fruity[e]
7	Ethyl heptanoate	1337	1331		×	MS, LRI	fruity[f]
8	Ethyl octanoate	1441	1435		×	MS, LRI	fruity, brandy[e]
9	Ethyl nonanoate	1533	1532		×	MS, LRI	fruity[e]
10	Ethyl decanoate	1640	1639		×	MS, LRI, STD	brandy, burnt, fruity[e]
11	Diethyl succinate	1689	1681		×	MS, LRI	fruity, melon[f]
12	Ethyl 9-decenoate	1698	1694		×	MS, LRI	fruity[f]
13	Ethyl phenylacetate	1786	1783		×	MS, LRI	fruity sweet[g]
14	Ethyl dodecanoate	1849	1843		×	MS, LRI, STD	floral, fruity, green apple[e]
15	Ethyl hexadecanoate	2257	2251		×	MS, LRI	wax[h]
Acetate esters							
16	Propyl acetate	974	973	×		MS, LRI	celery, floral, pear[e]
17	Octyl acetate	1475	1475	×		MS, LRI	citrus, fat, wood[e]
18	1-Phenylethyl acetate	1828	1813	×		MS, LRI	floral, fruit, honey[e]
19	Butyl acetate	1074	1074	×	×	MS, LRI, STD	apple, fruit, pungent[e]
20	Hexyl acetate	1275	1273	×	×	MS, LRI, STD	fruity[e]
21	3-Methylbutyl acetate	1123	1123		×	MS, LRI	apple, fruit, sweet[e]
22	2-Phenylethyl acetate	1814	1813		×	MS, LRI	flowery, fruit, honey[g]
Other esters							
23	Butyl butanoate	1223	1220	×		MS, LRI, STD	floral[e]
24	Butyl hexanoate	1413	1407	×		MS, LRI, STD	fruity, grass, green[e]
25	Hexyl butanoate	1415	1414	×		MS, LRI, STD	fruity, apple, fresh[e]
26	Hexyl 2-methylbutanoate	1433	1433	×		MS, LRI	strawberry[e]
27	Hexyl hexanoate	1610	1605	×		MS, LRI	apple, fruity[e]
28	Butyl 3-hydroxybutanoate	1690	1684	×	×	MS, LRI	fruity, brandy[e]
29	Methyl octanoate	1387	1385		×	MS, LRI	fruity, orange, sweet, wine[e]
Higher alcohols							
30	(2E)-2-hexen-1-ol	1409	1406	×		MS, LRI	grass[e]
31	1-Octen-3-ol	1451	1450	×		MS, LRI, STD	thyme[e]
32	2-Ethyl-1-hexanol	1492	1491	×		MS, LRI	citrus, green, rose[e]
33	2-Methyl-1-propanol	1095	1092	×	×	MS, LRI	apple, fusel, malt[e]
34	1-Butanol	1151	1142	×	×	MS, LRI	medicine, fruit[e]
35	1-Pentanol	1255	1250	×	×	MS, LRI	balsamic, fruity[e]
36	1-Hexanol	1357	1355	×	×	MS, LRI	green, herbaceous[e]
37	3-Octanol	1395	1393	×	×	MS, LRI	citrus, nut, oily[e]
38	6-Methyl-5-hepten-2-ol	1466	1465	×	×	MS, LRI	rose[e]
39	1-Octanol	1561	1557	×	×	MS, LRI, STD	chemical, metal, burnt[e]
40	3-Methyl-1-butanol	1215	1209		×	MS, LRI	alcohol, nail polish[e]
41	3-Ethoxy-1-propanol	1374	1373		×	MS, LRI	fruity[f]
42	3-(Methylthio)-1-propanol	1720	1719		×	MS, LRI	/
43	2-Phenylethanol	1918	1907		×	MS, LRI	honey, rose Floral[g]
Acids							
44	Hexanoic acid	1854	1846	×	×	MS, LRI, STD	sweat[e]
45	Nonanoic acid	2182	2170	×	×	MS, LRI	green, fat[h]
46	Acetic acid	1455	1449		×	MS, LRI	sour, vinegar-like[e]
47	2-Methylpropanoic acid	1575	1570		×	MS, LRI	rancid, butter, cheese[g]
48	Butanoic acid	1629	1624		×	MS, LRI	rancid[e]
49	Octanoic acid	2061	2060		×	MS, LRI	rancid, fatty[g]
50	Decanoic acid	2281	2276		×	MS, LRI	rancid fat[g]
51	2-Methylbutanoic acid	1666	1662		×	MS, LRI	/
Aldehydes							
52	Hexanal	1085	1083	×		MS, LRI, STD	grassy, green apple[e]
53	2-Hexenal	1218	1213	×		MS, LRI	green, grassy, pungent[g]
54	Acetaldehyde	745	702	×	×	MS, LRI	pungent, ripe apple[e]
55	4-Methylbenzaldehyde	1654	1648		×	MS, LRI	/
Ketones							
56	3-Hydroxy-2-butanone	1290	1285	×		MS, LRI	buttery, fatty[e]
57	6-Methyl hept-5-en-2-one	1340	1339	×	×	MS, LRI, STD	pungent[e]
58	2,6,8-Trimethyl-4-nonanone	1402	/	×	×	MS, LRI	/
Terpenes							
59	α-Farnesene	1747	1745	×		MS, LRI	wood sweet[g]
60	β-Damascenone	1837	1823	×	×	MS, LRI, STD	apple, rose, honey[g]
Monoterpenes							
61	Linalool	1548	1547	×	×	MS, LRI	citrus, floral, sweet, grape-like[f]
Lactones							
62	γ-Butyrolactone	1635	1632		×	MS, LRI	caramel, sweet[h]

(*continued on next page*)

Table 2 (*continued*)

Peak number[a]	Compounds	RI		Presence		Identification[c]	Odor descriptor[d]
		Measured[b]	Literature	Juice	Cider		
Acetals							
63	1-Ethoxy-1-methoxyethane	866	845		×	MS, LRI	fruity[e]
64	1-(1-ethoxyethoxy)pentane	1097	1098		×	MS, LRI	fruity, alcoholic[e]

[a] Number of volatile compounds identified.
[b] Kovat's retention indices of volatiles using DB-WAX Column.
[c] Identification, MS: mass spectrum; LRI: literature retention index; STD: standard.
[d] Odor descriptors based on literature.
[e] He et al., 2021.
[f] Liu et al., 2019.
[g] Wang et al., 2024.
[h] https://www.flavornet.org/flavornet.html.

Table 3
Average semi-quantified concentrations of volatile compounds in apple juice and corresponding cider samples fermented with *S. cerevisiae* and *T. delbrueckii*.

Samples	Ethyl esters	Acetate esters	Other esters	Higher alcohols	Acids	Aldehydes	Ketones	Terpenes	Lactones	Acetal
Juices										
T1J	0.07 ± 0.00 c	1.39 ± 0.17 b	0.03 ± 0.00 c	0.81 ± 0.11 d	0.05 ± 0.01 ab	0.49 ± 0.05 ab	0.04 ± 0.00 b	0.02 ± 0.00 c	ND	ND
T2J	0.37 ± 0.01 b	0.02 ± 0.00 c	0.02 ± 0.01 c	1.98 ± 0.10 a	0.05 ± 0.00 ab	0.23 ± 0.01 c	0.07 ± 0.00 a	0.05 ± 0.01 bc	ND	ND
ANJ	ND	2.59 ± 0.33 a	0.24 ± 0.05 b	1.84 ± 0.21 a	0.01 ± 0.01 b	0.61 ± 0.07 a	0.08 ± 0.01 a	0.08 ± 0.02 ab	ND	ND
ALJ	0.01 ± 0.00 c	1.63 ± 0.10 b	0.32 ± 0.02 b	1.33 ± 0.01 bc	0.08 ± 0.02 a	0.37 ± 0.01 bc	0.04 ± 0.00 b	0.06 ± 0.01 b	ND	ND
RAJ	0.09 ± 0.01 c	0.01 ± 0.00 c	0.83 ± 0.07 a	1.10 ± 0.10 cd	0.04 ± 0.01 ab	0.58 ± 0.05 a	0.08 ± 0.01 a	0.11 ± 0.02 a	ND	ND
MVJ	1.01 ± 0.09 a	0.01 ± 0.00 c	0.07 ± 0.01 c	1.61 ± 0.14 ab	0.03 ± 0.00 ab	0.32 ± 0.02 c	0.04 ± 0.00 b	0.02 ± 0.00 c	ND	ND
Mean	0.26 ± 0.36 C	0.94 ± 1.00	0.25 ± 0.28 A	1.44 ± 0.41 B	0.04 ± 0.02 C	0.43 ± 0.14	0.06 ± 0.02 C	0.06 ± 0.03 B	/	/
Ciders fermented by *S.cerevisiae*										
T1S	3.64 ± 0.60 c	0.62 ± 0.36 b	ND	15.38 ± 2.32	1.98 ± 0.42 b	0.57 ± 0.43	0.08 ± 0.01 b	0.12 ± 0.03 bc	0.01 ± 0.02	0.04 ± 0.06
T2S	6.07 ± 1.75 bc	0.35 ± 0.04 b	ND	18.95 ± 2.83	3.87 ± 0.41 a	0.56 ± 0.35	0.10 ± 0.02 b	0.19 ± 0.03 a	ND	0.03 ± 0.03
ANS	5.06 ± 2.59 bc	1.46 ± 0.72 a	ND	17.49 ± 3.65	4.11 ± 1.63 a	0.17 ± 0.14	0.14 ± 0.03 a	0.16 ± 0.07 abc	0.01 ± 0.00	0.01 ± 0.01
ALS	13.15 ± 5.69 abc	0.74 ± 0.25 b	0.04 ± 0.03 b	17.36 ± 2.84	5.51 ± 1.39 a	0.38 ± 0.32	0.10 ± 0.01 b	0.18 ± 0.04 ab	0.01 ± 0.01	0.05 ± 0.05
RAS	14.24 ± 3.37 ab	0.68 ± 0.21 b	0.01 ± 0.00 b	18.40 ± 3.04	3.99 ± 0.60 a	0.16 ± 0.12	0.15 ± 0.02 a	0.12 ± 0.01 c	ND	ND
MVS	20.44 ± 13.97 a	0.55 ± 0.36 b	0.07 ± 0.03 a	17.07 ± 6.32	4.78 ± 1.87 a	0.31 ± 0.18	0.13 ± 0.03 a	0.13 ± 0.05 bc	ND	0.03 ± 0.03
Mean	10.43 ± 6.00 A	0.73 ± 0.35	0.04 ± 0.02 B	17.44 ± 1.13 A	4.04 ± 1.08 A	0.36 ± 0.17	0.12 ± 0.03 A	0.15 ± 0.03 A	0.01 ± 0.01 B	0.03 ± 0.01
Ciders fermented by *T.delbrueckii*										
T1T	3.93 ± 0.79 bc	0.48 ± 0.30	ND	17.00 ± 1.38 bc	1.84 ± 0.56	0.19 ± 0.22 bc	0.07 ± 0.01 b	0.10 ± 0.02 c	0.01 ± 0.00 ab	ND
T2T	4.64 ± 1.28 b	0.44 ± 0.33	ND	21.24 ± 4.12 a	2.08 ± 0.58	0.30 ± 0.24 abc	0.10 ± 0.02 a	0.17 ± 0.06 a	0.01 ± 0.01 b	0.02 ± 0.02 bc
ANT	2.80 ± 0.90 c	0.48 ± 0.24	ND	17.28 ± 1.92 bc	1.90 ± 0.63	0.16 ± 0.10 bc	0.11 ± 0.01 a	0.13 ± 0.04 bc	0.01 ± 0.00 b	0.02 ± 0.02 abc
ALT	5.54 ± 1.34 b	0.52 ± 0.14	ND	15.38 ± 1.96 bc	2.51 ± 0.36	0.50 ± 0.34 a	0.08 ± 0.01 ab	0.17 ± 0.03 b	0.01 ± 0.01 ab	0.06 ± 0.04 a
RAT	8.46 ± 1.49 a	0.60 ± 0.09	ND	17.75 ± 2.37 ab	2.56 ± 0.42	0.05 ± 0.03 c	0.09 ± 0.01 ab	0.11 ± 0.02 bc	0.02 ± 0.02 a	ND
MVT	5.19 ± 1.38 b	0.30 ± 0.08	ND	13.89 ± 2.39 c	2.07 ± 0.73	0.41 ± 0.11 ab	0.10 ± 0.02 a	0.10 ± 0.03 c	0.01 ± 0.01 b	0.05 ± 0.03 ab
Mean	5.09 ± 1.75 B	0.47 ± 0.09	/	17.09 ± 2.27A	2.16 ± 0.28 B	0.27 ± 0.15	0.09 ± 0.01 B	0.13 ± 0.03 A	0.01 ± 0.01 A	0.04 ± 0.02

Results represent the mean ± standard deviation. Juice samples in triplicate, cider samples in nine replicates (3 biological replicates × 3 analytical replicates). ND: not detected. Statistically significant differences between cultivarss with each sample type are shown with lower case letter a-d and for mean values are shown with upper case letters A-C (One-way ANOVA test, Tukey's test and T-test; $p < 0.05$). Sample abbreviations refer to Table 1.

and ciders. They are primarily formed during fermentation through yeast metabolism and chemical esterification (He et al., 2022; Hinkley et al., 2023). Accordingly, a marked increase in ethyl esters was observed in ciders compared to juices. For instance, ethyl hexanoate rose from non-detectable levels in juices to 25.88%–28.53% of total ethyl esters in ciders, and ethyl acetate increased 72.41–97.85-fold. These results are consistent with previous studies on apple juice and pomace fermentation using SC, TD, *L. thermotolerans* and *S. pombe* (He et al.,

2021; Wang et al., 2024), confirming that fermentation substantially expands the ester profile beyond native juice volatiles.

Higher alcohols, which are primarily produced by yeast through the catabolism of amino acids via the Ehrlich pathway (Jagatić Korenika et al., 2022), became the most abundant group of VOC in ciders (except MVS), with total content increasing by 8.64–20.89 times after fermentation. The profile was dominated by 3-methyl-1-butanol and 2-phenylethanol, consistent with previous research involving yeast and *Oenococcus oeni* in cider fermentation (Li et al., 2020). Together, these two compounds accounted for over 80% of total higher alcohols. A third notable alcohol, 1-hexanol, constituted about 10% of the total in cider samples. These alcohols are associated with *floral*, *herbaceous* or *earthy* odor (Romano et al., 2022; Yang et al., 2021). The highest total level of higher alcohols was observed in T2T, indicating a richer alcohol aroma profile and highlights the suitability of T2 for cider production.

Volatile acids produced from both apple precursors and yeast fatty acid metabolism were also strongly affected by fermentation (Gallart et al., 1997; Swiegers et al., 2005). Accordingly, while only hexanoic acid and nonanoic acid were detected in juices, six additional acids (acetic acid, 2-methylpropanoic acid, butanoic acid, octanoic acid, decanoic acid and 2-methylbutanoic acid) appeared after fermentation. These acids contribute to the diverse aromas of ciders, including *vinegar-like sharpness, sweetness,* and slightly *off* notes (He et al., 2021; Hu et al., 2018). Octanoic acid dominated the acid fraction in all cider samples, accounting for 44.74% of total acids in SC-fermented ciders and 20.17% in TD-fermented ciders. Overall, total volatile acids were approximately twice as high in SC-fermented ciders as in TD-fermented ciders, consistent with reported metabolic differences between these yeasts (Jiang et al., 2020; Wang et al., 2003).

Aldehydes are common intermediates in yeast sugar metabolism and are often rapidly converted into alcohols, acids or acetals during fermentation (Liu et al., 2019; Swiegers et al., 2005). They were present at lower levels, with four compounds identified across samples. Hexanal and 2-hexenal were exclusive to juices, consistent with Wang et al. (2024), who reported their disappearance after alcoholic fermentation of apple pomace with different yeast strains. Acetaldehyde was the only aldehyde detected in both juices and ciders, and its concentration increased after fermentation. It typically associated with pleasant *nut* and *dried fruits* aromas (Xu et al., 2025). Lactones and acetals are secondary fermentation products formed from fatty acids and aldehydes in the presence of alcohols during fermentation (Liu et al., 2019). They were detected exclusively in ciders, acting as additional fermentation markers that enhance aromatic complexity with *fruity* and *floral* notes (Niu et al., 2019; Qin et al., 2018; Sah et al., 2024).

PCA was performed to visualize overall differences in volatile profiles between juices (n = 18 = 6 × 3 analytical replicates) and ciders (SC-

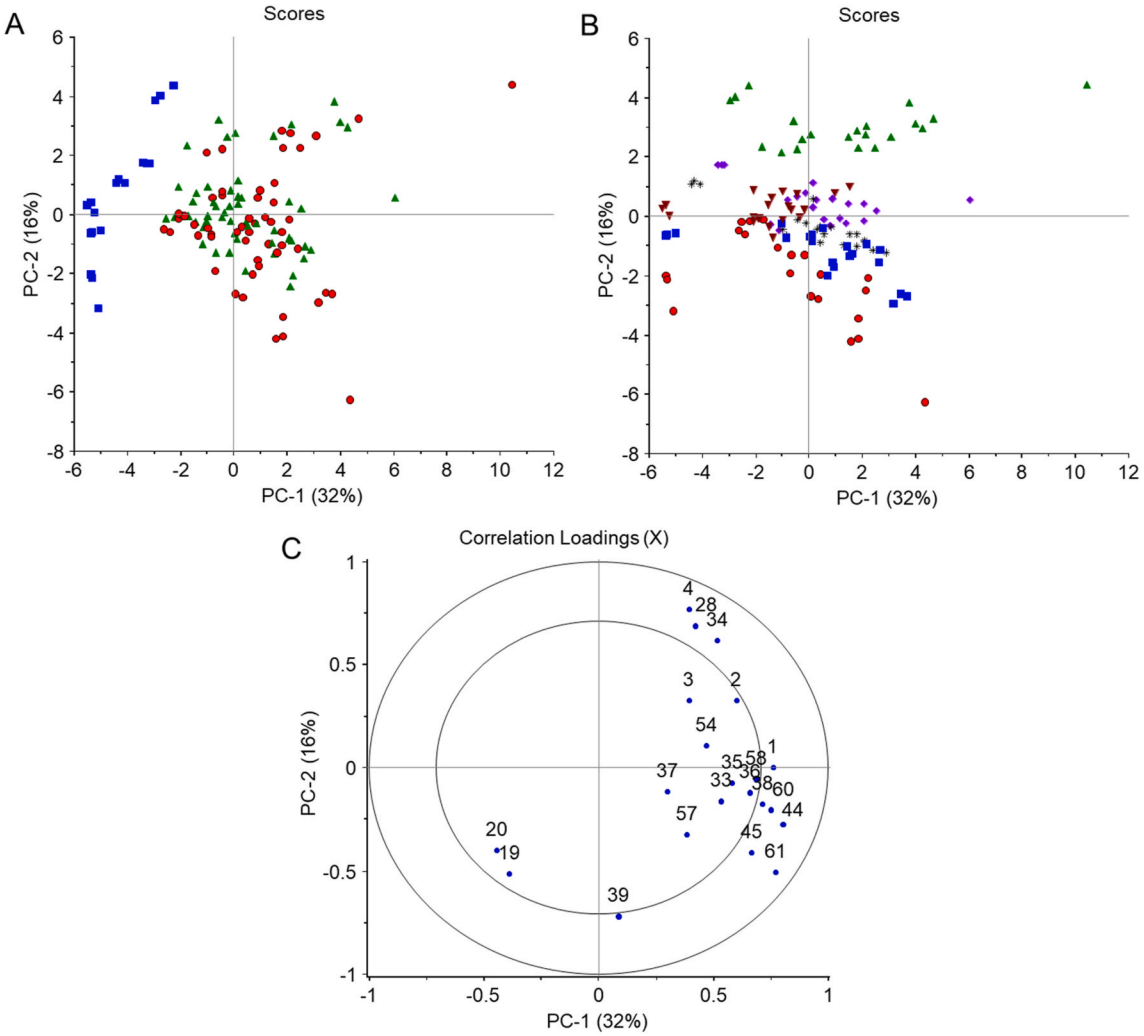

Fig. 4. Principal Component Analysis (PCA) of all samples (n = 126) based on selected volatile compounds (n = 21) observed in all sample types (juices and ciders). A. Scores plot of the model labelled according to sample type (juices blue rectangles, *S. cerevisiae* ciders red circles, *T. delbrueckii* ciders green triangles); B. Same scores plot labelled according to apple cultivar: 'Tunnistamaton1', brown inverted triangles; 'Tunnistamaton2', violet diamonds; 'Antonovka', red circles; 'Aleksanteri', blue rectangles; 'Rambo', black stars; and 'Mustialan Iso Venäläinen', green triangles; C. volatile compound variables; numbers refer to Table 2.

and TD-ciders: n = 54 = 6 × 3 biological replicates × 3 analytical replicates), using VOC (n = 21) detected in all samples as variables (Fig. 4). The 21 selected VOC included seven esters, seven alcohols, two acids, one aldehydes, two ketones and two tarpenes. The first two PCs accounted for 48% of the total variance (32% and 16%, respectively). PC-1 clearly separated juices from ciders, underscoring fermentation as the primary driver of volatile composition change. PC-2 revealed clustering patterns according to apple cultivar, indicating that cultivar-specific traits also significantly shape the final aromatic profile For instance, ANJ was closely associated with butyl acetate and hexyl acetate, whereas MVS and MVT were linked to ethyl 3-hydroxybutanoate. To better understand these associations, further analyses were performed.

3.4.2. *Impact of yeast strain and apple cultivar on the volatile profile*

The separation of cider samples fermented with SC and TD (Y-data, n = 2) based on volatile composition (X-data, n = 35) was analyzed using a PLS-DA model (Fig. 5). The model clearly separated ciders according to the fermenting yeast strain (SC vs. TD), highlighting strain-specific influences on VOC formation, including esters, alcohols, and acids (Ferremi Leali et al., 2024). According to the correlation loadings plot, 3-ethoxy-1-propanol was strongly associated with TD, being detected in all TD-fermented, whereas it was present only at trace levels in SC-fermented ciders and was detected solely in MVS. This compound has been reported to contribute *fruity* aromas (Liu et al., 2019), and its average relative concentration in TD-fermented ciders was 12.79-fold higher than in MVS. Similar observations have been reported for bilberry wines, where TD fermentation produced higher levels of 3-ethoxy-1-propanol than *S. pombe* strains (Liu et al., 2019). In contrast, esters were more strongly associated with SC fermentation, consistent with previous studies showing that SC generally produces higher ester levels than non-*Saccharomyces* species (Benito et al., 2019; Lorenzini et al., 2019). For instance, the relative concentration of ethyl octanoate in MVS was 11.11-fold higher than in MVT, and methyl octanoate was detected exclusively in SC-fermented ciders.

To further elucidate the combined effects of apple cultivar and yeast strain on cider aroma profiles, three additional PCA models were constructed (Fig. 6). In the PCA using only juice samples (Fig. 6A), the first two PCs explained 64% of the total variance. Distinct clustering patterns were observed: RAJ, ANJ, and MVJ emerged as the major contributors to juice VOC profiles. RAJ displayed the lowest variability and formed a tight cluster characterized by butyl butanoate, hexyl butanoate and hexyl 2-methylbutanoate. In contrast, ANJ and MVJ were associated with a broader range of volatiles groups: ANJ correlated with esters, higher alcohols, aldehydes, and terpenes, whereas MVJ was associated with esters, higher alcohols, ketones, and aldehydes. These differences reflect cultivar-specific traits and highlight the foundamental role of the juice matrix in shaping the volatile profile during fermentation (Guo et al., 2020; Jagatić Korenika et al., 2022). Skin color also appears to influence ester composition: the yellow-skinned ANJ was associated with higher levels of 1-phenylethyl acetate, butyl acetate and hexyl acetate, whereas the red-skinned RAJ showed higher levels of butyl butanoate and hexyl butanoate. These findings agree with Dixon and Hewett (2000), who reported that yellow-skinned cultivars tend to produce more acetic acid esters, while red-skinned cultivars are more closely associated with butyric acid esters. Importantly, the concentrations of these esters exceeded their reported odor detection thresholds (Coelho et al., 2021), indicating that the differences observed between cultivars are likely to be sensory relevance.

Fig. 6B illustrates the combined influence of apple cultivar and SC fermentation on cider aroma profiles (cider n = 18 = 6 × 3 biological replicates; VOC n = 35). The first two PCs explained 45% of the total variance. ALS and RAS were located close together in the correlation loadings plot, indicating similar volatile characteristics. However, ALS was more strongly associated with ethyl 3-methylbutanoate, whereas RAS was more related to alcohols, acids and acetals (1-pentanol, butanoic acid and 1-(1-ethoxyethoxy)pentane). ANS was associated with butyl acetate and hexyl acetate, which have been reported to contribute *fruity* and *sweet* notes (Chitarrini et al., 2020). MVS showed the strongest association with ester production, especially ethyl 2-methylbutanoate, ethyl 3-hydroxybutanoate, ethyl heptanoate and ethyl nonanoate, which are known to *fruity*, *grape* and *roasted nut* aromas (He et al., 2021; Liu et al., 2019). Interestingly, although RAJ contained 2.81-fold more total sugar content than MVJ, MVS yielded the highest total ester concentration in all ciders, 1.41-fold higher than RAS. This indicates that sugar content alone does not fully explain the observed differences in ester formation among cultivars. Other factors, including assimilable nitrogen availability, fatty acid synthesis, osmotic stress at fermentation onset and ester hydrolysis, have been reported to influence ester production (Seguinot et al., 2020; Tocci et al., 2023).

Fig. 6C presents the combined effect of apple cultivar and TD fermentation (cider n = 18 = 6 × 3 biological replicates; VOC n = 34, excluding methyl octanoate, which was detected only in SC-fermented ciders). The first two PCs explained 44% of the total variance. RAT,

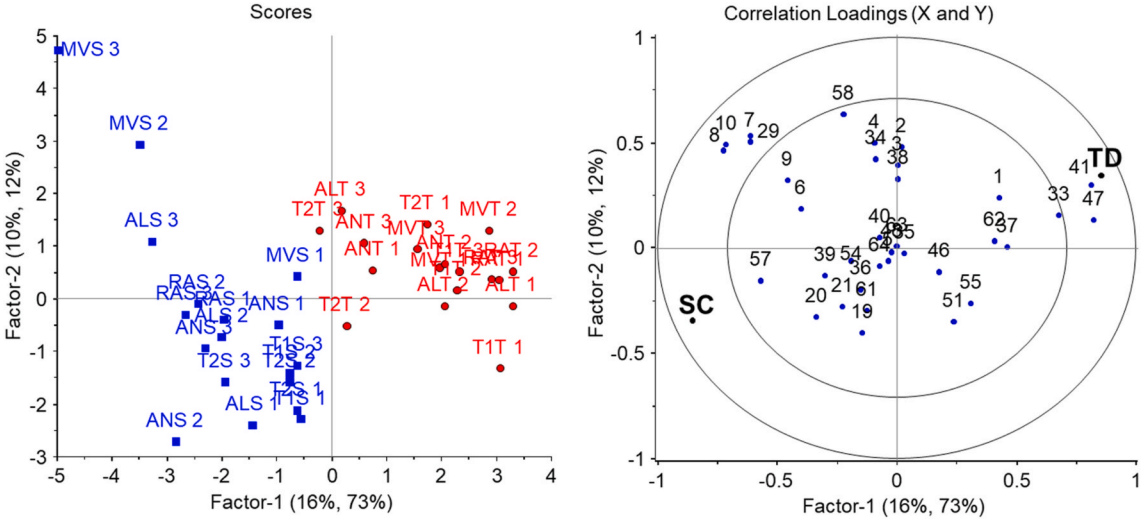

Fig. 5. Partial Least Squares Discrimination Analysis (PLS-DA) model of volatile compound variables as X-data (n = 35) and cider type as Y-data (n = 2; S: *S. cerevisiae* ciders with blue rectangles; T: *T. delbrueckii* ciders with red circles). Triplicate analytical replicates have been averaged to biological replicates (numbers 1-3). Cultivar abbreviations refer to Table 1; volatile compound variable numbers refer to Table 2.

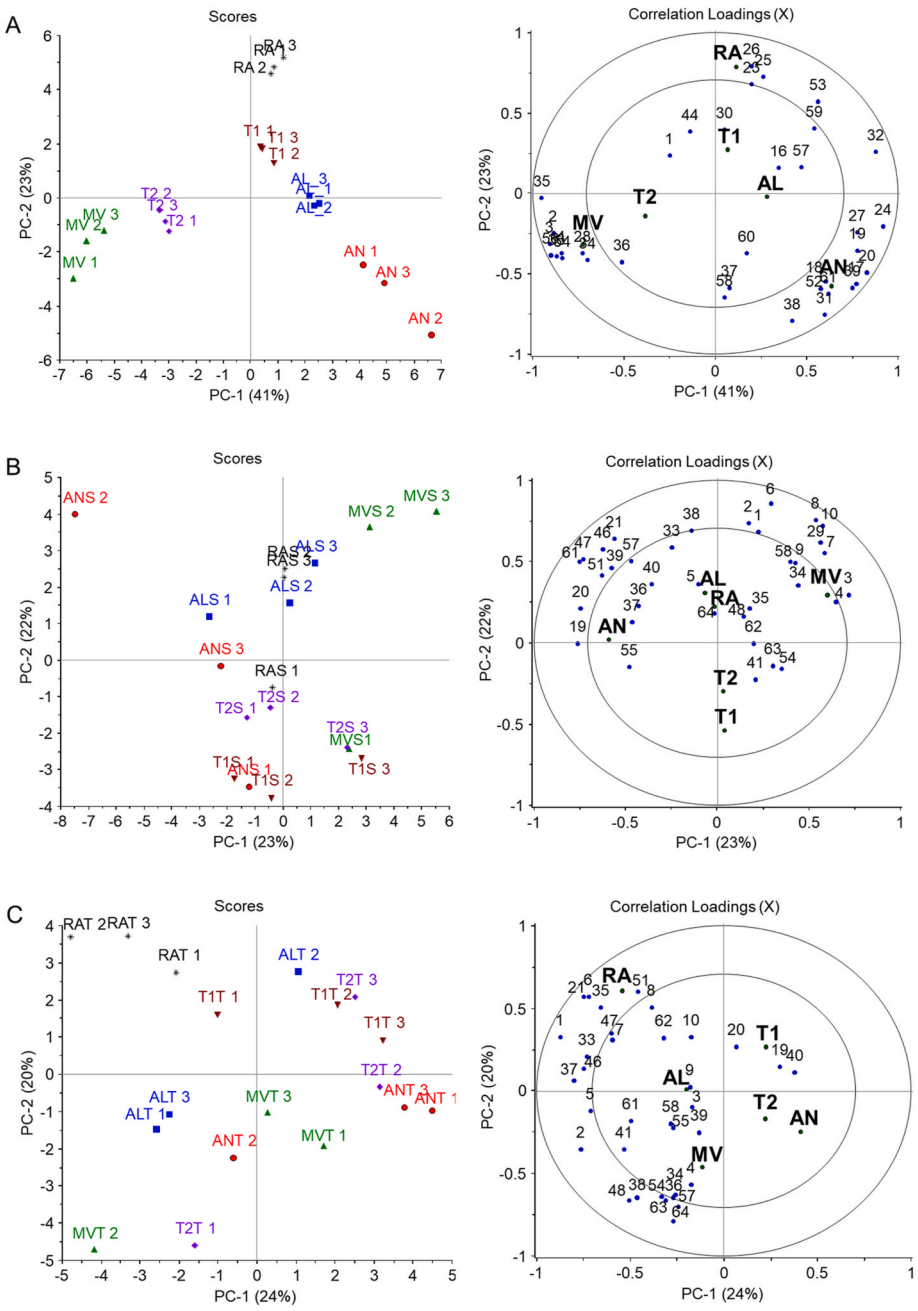

Fig. 6. Three Principal Component Analysis (PCA) models of volatile compound variables in different sample types. A. juice samples (n = 18, 35 variables); B. *S. cerevisiae* ciders (n = 18, 35 variables); C. *T. delbrueckii* ciders (n = 18, 34 variables). For models B and C, the triplicate analytical replicates have been averaged to biological replicates (numbers 1-3); sample abbreviations refer to Table 1; volatile compound variable numbers refer to Table 2.

ALT and MVT were identified as the main contributors to volatile variation. RAT exhibited strong associations with ethyl hexanoate, ethyl octanoate, 3-methylbutyl acetate, and showed the highest total ester concentration, 1.50–2.76 times of other TD-fermented ciders. Nevertheless, the relatively high sugar level in RAT may still have contributed to its elevated ester concentration by providing additional fermentable substrates (Lin et al., 2025; Ruppert et al., 2021). ALT was associated with ethyl 2-methylbutanoate and ethyl nonanoate, whereas MVT showed the highest concentration of ethyl 3-hydroxybutanoate. Furthermore, T1T was closely associated with butyl acetate and hexyl acetate and the higher alcohol 3-methyl-1-butanol. This higher alcohol, which was not detected in juices, increased markedly during fermentation via amino acids deamination and decarboxylation, becoming the dominant alcohol in ciders and has been linked to *whiskey* and *burnt* aromas (Qin et al., 2018). Among all ciders, its relative concentration was highest in T1T, approximately 1.91-fold higher than in MVT, which exhibited the lowest level.

Overall, these PCA analyses indicate that the apple cultivar provides the primary chemical template that shapes the final volatile composition of ciders, while the yeast strain modulates specific metabolic pathways to adjust the profile.

4. Conclusion

This study demonstrated that both apple cultivar and yeast selection (*S. cerevisiae* v.s. *T. delbrueckii*) significantly shaped the chemical and volatile aroma profiles of ciders produced from traditional apple resources. Among the cultivars studied, ‘Rambo’ exhibited the greatest

potential for cider production primarily due to its favourable juice composition. Within the framework defined by each cultivar, yeast selection further fine tuned the volatile organic compound profile: *T. delbrueckii* favored higher alcohols and specific esters known to contribute to floral, fruity, and complex alcoholic notes, whereas *S. cerevisiae* promoted esters and volatile acids formation, likely enhancing fruity, sour, and layered aroma profiles. Promising synergies were identified in specific cultivar–yeast combination, such as 'Rambo' with *T. delbrueckii* and 'Aleksanteri', 'Antonovka', or 'Mustialan Iso Venäläinen' with *S. cerevisiae*, which produced distinct volatile profiles that may enhance the overall aroma complexity of the ciders. This work provides a basis for developing high-quality ciders from heritage and underutilized traditional apple cultivars grown in old orchards in Finland.

However, the biological variability observed among replicates (Fig. 6B and C) highlights the sensitivity of fermentation outcomes to experimental conditions. To build robust and applicable production protocols, future studies should prioritize controlled anaerobic fermentation systems with aseptic sampling to minimize oxidation risks and metabolite fluctuations, thereby improving reproducibility and clarifying the respective roles of cultivar and yeast. Furthermore, integrating sensory analysis and expanding trials to multiple harvest years and locations are essential next steps to confirm the practical potential of these interactions and to account for natural agricultural variation. This integrated approach can support targeted cider design and the valorization of local apple genetic resources.

CRediT authorship contribution statement

Qizai Wang: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Oskar Laaksonen:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation. **Mahsa Sadat Jafari:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Ada Obianuju Okwum:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Annelie Damerau:** Writing – review & editing, Supervision, Methodology, Data curation. **Maarit Heinonen:** Writing – review & editing, Resources. **Wenjia He:** Writing – review & editing, Methodology. **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 the following financial interests/personal relationships which may be considered as potential competing interests: Qizai Wang reports financial support was provided by Niemi Foundation. Qizai Wang reports financial support was provided by the Lieto Savings Bank Foundation. Baoru Yang reports financial support was provided by Research Council of Finland research infrastructure funding. Baoru Yang reports financial support was provided by the European Union-NextGenerationEU instrument funding. Baoru Yang reports financial support was provided by the Finland-China Food and Health Network. Wenjia He reports financial support was provided by the China Scholarship Council. Wenjia He reports financial support was provided by the First division Alar City Science and Technology Project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2026.108699.

Data availability

Data will be made available on request.

References

Al Daccache, M., Koubaa, M., Maroun, R. G., Salameh, D., Louka, N., & Vorobiev, E. (2020). Impact of the physicochemical composition and microbial diversity in apple juice fermentation process: A Review. *Molecules, 25*(16), 3698. https://doi.org/10.3390/molecules25163698

Belda, I., Ruiz, J., Beisert, B., Navascués, E., Marquina, D., Calderón, F., Rauhut, D., Benito, S., & Santos, A. (2017). Influence of *Torulaspora delbrueckii* in varietal thiol (3-SH and 4-MSP) release in wine sequential fermentations. *International Journal of Food Microbiology, 257*, 183–191. https://doi.org/10.1016/j.ijfoodmicro.2017.06.028

Benito, Á., Calderón, F., & Benito, S. (2019). The Influence of non-*Saccharomyces* species on wine fermentation quality parameters. *Fermentation, 5*(3), 54. https://doi.org/10.3390/fermentation5030054

Bingman, M. T., Hinkley, J. L., Bradley, C. P., & Cole, C. A. (2021). Aroma profiles of dry-hopped ciders produced with citra, galaxy, and mosaic hops. *Applied Sciences, 12*(1), 310. https://doi.org/10.3390/app12010310

Călugăr, P. C., Coldea, T. E., Pop, C.-R., Stan, L., Socaci, S. A., Ranga, F., Hegheș, S. C., Geană, E.-I., & Mudura, E. (2024). Effect of co-inoculation of *Saccharomyces* and non-*Saccharomyces* yeasts and nutrients addition during malolactic fermentation on apple cider composition. *Food Bioscience, 60*, Article 104314. https://doi.org/10.1016/j.fbio.2024.104314

Castro, L. F., Affonso, A. D., & Perry, K. P. (2025). Effect of apple pomace addition during fermentation on the phenolic content, chemical composition, and sensory properties of cider. *Beverages, 11*(4), 95. https://doi.org/10.3390/beverages11040095

Chitarrini, G., Dordevic, N., Guerra, W., Robatscher, P., & Lozano, L. (2020). Aroma investigation of nnew and standard apple varieties grown at two altitudes using Gas Chromatography-Mass Spectrometry combined with sensory analysis. *Molecules, 25*(13), 3007. https://doi.org/10.3390/molecules25133007

Coelho, E., Pinto, M., Bastos, R., Cruz, M., Nunes, C., Rocha, S. M., & Coimbra, M. A. (2021). Concentrate apple juice industry: Aroma and pomace valuation as food ingredients. *Applied Sciences, 11*(5), 2443. https://doi.org/10.3390/app11052443

Dixon, J., & Hewett, E. W. (2000). Factors affecting apple aroma/flavour volatile concentration: A Review. *New Zealand Journal of Crop and Horticultural Science, 28*(3), 155–173. https://doi.org/10.1080/01140671.2000.9514136

European Cider and Fruit Wine Association (AICV), & GlobalData. (2024). *European cider trends 2024* [PDF presentation]. AICV https://aicv.org/files/attachments/.594/AICV_Cider_Trends_2024.pdf. (Accessed 1 May 2025).

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), 712. https://doi.org/10.3390/jof7090712

Ferremi Leali, N., Salvetti, E., Luzzini, G., Salini, A., Slaghenaufi, D., Fusco, S., Ugliano, M., Torriani, S., & Binati, R. L. (2024). Differences in the volatile profile of apple cider fermented with *Schizosaccharomyces pombe* and *Schizosaccharomyces japonicus*. *Fermentation, 10*(3), 128. https://doi.org/10.3390/fermentation10030128

Gallart, M., Francioli, S., Viu-Marco, A., López-Tamames, E., & Buxaderas, S. (1997). Determination of free fatty acids and their ethyl esters in musts and wines. *Journal of Chromatography A, 776*(2), 283–291. https://doi.org/10.1016/S0021-9673(97)00383-X

Garkava-Gustavsson, L., Mujaju, C., Sehic, J., Zborowska, A., Backes, G. M., Hietaranta, T., & Antonius, K. (2013). Genetic diversity in Swedish and Finnish heirloom apple cultivars revealed with SSR markers. *Scientia Horticulturae, 162*, 43–48. https://doi.org/10.1016/j.scienta.2013.07.040

Guo, J., Yue, T., Yuan, Y., Sun, N., & Liu, P. (2020). Characterization of volatile and sensory profiles of apple juices to trace fruit origins and investigation of the relationship between the aroma properties and volatile constituents. *LWT, 124*, Article 109203. https://doi.org/10.1016/j.lwt.2020.109203

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*, Article 131437. 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*, Article 128833. https://doi.org/10.1016/j.foodchem.2020.128833

Heinonen, M., & Bitz, L. (2019). How to discover traditional varieties and shape in a national germplasm collection: The case of Finnish seed born apples (*Malus* × *domestica* Borkh.). *Sustainability, 11*(24), 7000. https://doi.org/10.3390/su11247000

Hinkley, J. L., Bingman, M. T., Lee, J. S., Bradley, C. P., & Cole, C. A. (2023). Volatile profile survey of five apple varieties grown in Southwest Colorado from juice to finished, dry-hopped cider. *Journal of the American Society of Brewing Chemists, 81*(1), 131–140. https://doi.org/10.1080/03610470.2021.2013645

Hu, K., Jin, G.-J., Mei, W.-C., Li, T., & Tao, Y.-S. (2018). Increase of medium-chain fatty acid ethyl ester content in mixed *H. uvarum/S. cerevisiae* fermentation leads to wine fruity aroma enhancement. *Food Chemistry, 239*, 495–501. https://doi.org/10.1016/j.foodchem.2017.06.151

Jagatić Korenika, A.-M., Preiner, D., Tomaz, I., Skendrović Babojelić, M., & Jeromel, A. (2022). Aroma profile of monovarietal Pét-Nat ciders: The role of croatian traditional apple varieties. *Horticulturae, 8*(8), 689. https://doi.org/10.3390/horticulturae8080689

Jiang, X., Lu, Y., & Liu, S. Q. (2020). Effects of pectinase treatment on the physicochemical and oenological properties of red dragon fruit wine fermented with *Torulaspora delbrueckii*. *LWT, 132*, Article 109929. https://doi.org/10.1016/j.lwt.2020.109929

Kelanne, N., Yang, B., Liljenbäck, L., & Laaksonen, O. (2020). Phenolic compound profiles in alcoholic black currant beverages produced by fermentation with *Saccharomyces* and non- *Saccharomyces* Yeasts. *Journal of Agricultural and Food Chemistry, 68*(37), 10128–10141. https://doi.org/10.1021/acs.jafc.0c03354

Li, Y., Liang, H., Wu, Y., Lin, X., Dai, Y., & Ji, C. (2026). Microbial interactions and sequential inoculation of Metschnikowia koreensis and *Saccharomyces cerevisiae* shape cider flavor development. *Food Bioscience, 75*, Article 108196. https://doi.org/10.1016/j.fbio.2025.108196

Li, C. X., Zhao, X. H., Zuo, W. F., Zhang, T. L., Zhang, Z. Y., & Chen, X. S. (2020). The effects of simultaneous and sequential inoculation of yeast and autochthonous *Oenococcus oeni* on the chemical composition of red-fleshed apple cider. *LWT, 124*, Article 109184. https://doi.org/10.1016/j.lwt.2020.109184

Lin, Y., Warmund, M. R., & Kwasniewski, M. T. (2025). Characterization of the volatile composition of fermented ciders made from dessert apple cultivars with and without maceration. *Journal of the American Society of Brewing Chemists, 83*(1), 101–116. https://doi.org/10.1080/03610470.2024.2319931

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*, 25–39. 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. *LWT, 99*, 224–230. https://doi.org/10.1016/j.lwt.2018.09.075

Mbuyane, L. L., De Kock, M., Bauer, F. F., & Divol, B. (2018). *Torulaspora delbrueckii* produces high levels of C5 and C6 polyols during wine fermentations. *FEMS Yeast Research, 18*(7). https://doi.org/10.1093/femsyr/foy084

Medina, S., Perestrelo, R., Pereira, R., & Câmara, J. S. (2020). Evaluation of volatilomic fingerprint from apple fruits to ciders: A useful tool to find putative biomarkers for each apple variety. *Foods, 9*(12), 1830. https://doi.org/10.3390/foods9121830

Niu, Y., Wang, P., Xiao, Z., Zhu, J., Sun, X., & Wang, R. (2019). Evaluation of the perceptual interaction among ester aroma compounds in cherry wines by GC–MS, GC–O, odor threshold and sensory analysis: An insight at the molecular level. *Food Chemistry, 275*, 143–153. https://doi.org/10.1016/j.foodchem.2018.09.102

Payan, C., Gancel, A.-L., Jourdes, M., Christmann, M., & Teissedre, P.-L. (2023). Wine acidification methods: A review. *OENO One, 57*(3), 113–126. https://doi.org/10.20870/oeno-one.2023.57.3.7476

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*, 713–723. https://doi.org/10.1016/j.foodres.2017.12.003

Renault, P., Coulon, J., Moine, V., Thibon, C., & Bely, M. (2016). Enhanced 3-Sulfanylhexan-1-ol production in sequential mixed fermentation with *Torulaspora delbrueckii/Saccharomyces cerevisiae* reveals a situation of synergistic interaction between two industrial strains. *Frontiers in Microbiology, 7*. https://doi.org/10.3389/fmicb.2016.00293

Romano, P., Braschi, G., Siesto, G., Patrignani, F., & Lanciotti, R. (2022). Role of yeasts on the sensory component of wines. *Foods, 11*(13), 1921. https://doi.org/10.3390/foods11131921

Ruppert, V., Innerhofer, G., Voit, J., Hiden, P., & Siegmund, B. (2021). The impact of the fermentation strategy on the flavour formation of Ilzer Rose (*Malus domestica Borkh.*) Apple Wine. *Foods, 10*(10), 2348. https://doi.org/10.3390/foods10102348

Sah, S., Putatunda, S., Majumder, R., & Das, A. (2024). Changes of volatile flavored compounds during lactic acid fermentation in cereal-based products. *Functional Foods in Health and Disease, 14*(11), 871–882. https://doi.org/10.31989/ffhd.v14i11.1503

Seguinot, P., Ortiz-Julien, A., & Camarasa, C. (2020). Impact of nutrient availability on the fermentation and production of aroma compounds under sequential inoculation eith *M. pulcherrima* and *S. cerevisiae*. *Frontiers in Microbiology, 11*, 305. https://doi.org/10.3389/fmicb.2020.00305

Sousa, A., Vareda, J., Pereira, R., Silva, C., Câmara, J. S., & Perestrelo, R. (2020). Geographical differentiation of apple ciders based on volatile fingerprint. *Food Research International, 137*, Article 109550. https://doi.org/10.1016/j.foodres.2020.109550

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A., & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research, 11*(2), 139–173. https://doi.org/10.1111/j.1755-0238.2005.tb00285.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), 4015. 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), 165. https://doi.org/10.3390/fermentation9020165

Wang, X. D., Bohlscheid, J. C., & Edwards, C. G. (2003). Fermentative activity and production of volatile compounds by *Saccharomyces* grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid. *Journal of Applied Microbiology, 94*(3), 349–359. https://doi.org/10.1046/j.1365-2672.2003.01827.x

Wang, X., Fan, G., Peng, Y., Xu, N., Xie, Y., Zhou, H., Liang, H., Zhan, J., Huang, W., & You, Y. (2023). Mechanisms and effects of non-*saccharomyces* yeast fermentation on the aromatic profile of wine. *Journal of Food Composition and Analysis, 124*, Article 105660. https://doi.org/10.1016/j.jfca.2023.105660

Wang, Q., Laaksonen, O., Xifre Pujol, E., Heinonen, M., Yang, B., & Kelanne, N. (2024). Impact of yeast selection on composition of vinegar fermented from pomace of a Finnish apple cultivar. *Food Bioscience, 62*, Article 105447. https://doi.org/10.1016/j.fbio.2024.105447

Xu, K., Liu, W., Zhao, Z., Wang, X., Sun-Waterhouse, D., & Guo, M. (2025). The physicochemical characteristics, volatile profiles and sensory attributes of the ciders made with juices or corresponding pomaces from different apple cultivars. *Food Chemistry, 488*, Article 144712. https://doi.org/10.1016/j.foodchem.2025.144712

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), 1051. https://doi.org/10.3390/foods10051051

Zeng, C., Mu, Y., Yuan, J., Zhang, H., Song, J., & Kang, S. (2025). Apple cultivar-influenced differences in cider: A comprehensive analysis of physicochemical and aroma compounds. *Microchemical Journal, 208*, Article 112382. https://doi.org/10.1016/j.microc.2024.112382