applied sciences

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Torulaspora delbrueckii Strain Behaviour within Different Refermentation Strategies for Sparkling Cider Production

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Abstract: *Torulaspora delbrueckii* is known for improving the aroma quality in wine and beer, but information for cider manufacturing is scarce. We explored the behaviour of two commercial strains of *T. delbrueckii* in apple juice fermentation to produce cider and sparkling cider. The influence of the strain, method, and strain–method interaction on the physicochemical parameters of cider was analysed by enzymatic and chromatographic assays. The data were subjected to an analysis of variance and a principal component analysis. Both strains also showed regular fermentation kinetics under pressure. Ethanol, lactic acid, acetic acid, and glycerol were produced with significant differences between strains and production methods. Then, 26 volatile compounds were identified, with higher alcohols being the quantitatively most abundant group. Strain A was associated with a higher production of methyl butanol, 2-phenyl ethanol, and butyric, propionic, and succinic acid esters, while strain B was associated with higher amounts of hexanoic acid, and acetate and valerate esters. In addition, 13 compounds showed significant differences between methods, and 14 were influenced by the method–strain interaction. Our findings encourage the further investigation of the application of *T. delbrueckii* as a solo player to produce ciders with a unique flavour.

Keywords: cider; *Torulaspora delbrueckii*; sparkling cider; Charmat method; Champenoise method; volatilome

Citation: Tocci, N.; Egger, M.; Hoellrigl, P.; Sanoll, C.; Beisert, B.; Brezina, S.; Fritsch, S.; Schnell, S.; Rauhut, D.; Conterno, L. *Torulaspora delbrueckii* Strain Behaviour within Different Refermentation Strategies for Sparkling Cider Production. *Appl. Sci.* **2023**, *13*, 4015. https://doi.org/10.3390/app13064015

Academic Editor: Ioannis G. Roussis

Received: 7 February 2023
Revised: 16 March 2023
Accepted: 20 March 2023
Published: 21 March 2023

1. Introduction

Cider is an alcoholic beverage obtained from the complete or partial fermentation of apple juice. It represents an important segment of the apple fruit industry in Europe, North America, and Australia, where apple cultivation is diffused [1]. The main cider-producing countries (UK, Spain, France, Ireland, and Germany) have long-standing cider styles and precise legislation. In the past, cider consumption was restricted to traditional regions while, nowadays, cider and sparkling ciders are becoming more popular and attractive for many consumers [2]. The increasing demand for ciders is accompanied by a growing request for natural and locally produced beverages with peculiar aromatic characteristics [3].

The use of a selected *Saccharomyces cerevisiae* starter is widespread in the industrial production to obtain a complete alcoholic fermentation, to avoid sensory deviation and to ensure a consistent and reproducible quality [4]. Nevertheless, in traditional and small-scale cider manufacturers, the fermentation is often spontaneous and carried out by indigenous yeasts [5]. The recognition of 'territoriality' is important for cider appreciation [4] and the choice of selected yeast starters may actively contribute to the expression of cider typicity. Non-*Saccharomyces* yeasts have often been considered as sources of spoilage and associated with higher levels of residual sugars and unpredictable by-products and off-flavours [6].

More recently, the significant role of non-*Saccharomyces* yeasts in enhancing the flavour, body, and texture complexity of fermented beverages have been revalued. Indigenous yeasts have been associated with the production of peculiar aroma compounds, such as esters, aldehydes, higher alcohols, acids, terpenes, as well as higher glycerol concentration [7].

The spontaneous fermentation of apple juice has been described as a complex of microbial activities characterized by the sequential enrichment of non-*Saccharomyces* and *Saccharomyces* yeasts. In the first phases of the process, *Kloeckera* spp., *Candida* spp., *Pichia* spp., *Hansenula* spp., *Hanseniaspora* spp., and *Metschnikowia* spp. represent the most detected yeasts, while *Saccharomyces cerevisiae* become dominant later when the ethanol produced by the early fermenting species inhibits their own growth [4]. In the recent years, several studies on the use of non-*Saccharomyces* yeasts for cider production have been published, suggesting an increasing interest in the search of novel actors to produce ciders with unique aromatic characteristics [8–12]. The influence of non-*Saccharomyces* yeasts has been investigated both in pure and in mixed fermentations, and in combination with *S. cerevisiae* [13–15]. Several studies documented the improvement of the fruity aroma of ciders correlated to the production of esters, higher alcohols, and terpenes with fruity aromas after a metabolomic analysis [16,17].

Among non-*Saccharomyces* yeasts, the use of *Torulaspora delbrueckii* is attracting the attention of wine, beer, and cider producers because of its good fermentation performance [18]. In a study on synthetic media, Ogawa et al. [19] described that *T. delbrueckii* produced metabolites correlated to odor activity. *T. delbrueckii* was associated with the valuable production of aromatic compounds such as fruity esters, lactones, thiols, aldehydes, and terpenes, contributing to a fruity and pastry bouquet, and the decreased amount of undesired aromatic compounds. Moreover, it has been documented that *T. delbrueckii* improves the quality of wine, resulting in a product characterized by a lower volatile acidity and acetaldehyde production, increased amount of glycerol, and greater mannoprotein and polysaccharide release in comparison with wine produced with *S. cerevisiae* [20,21].

Despite non-*Saccharomyces* yeasts having been investigated as main actors in cider production, only a few publications are available on the application of *T. delbrueckii* in cider making [9,11,19,22].

The aim of the present study was to investigate the fermentative behaviour of two different strains of *T. delbrueckii* in producing cider and sparkling cider in using different process methods. The impact of the yeast strain, methods, and strain–method interaction on the chemical profile and sensorial perception was evaluated through headspace solid-phase micro-extraction gas chromatography–mass spectrometry (HS-SPME-GC-MS), and sensory tasting performed by a panel of semi-trained experts. The collected data were analysed by applying a principal component analysis (PCA) and analysis of variance (ANOVA).

2. Materials and Methods

2.1. Micro-Organisms

Torulaspora delbrueckii ZYMAFLORE® ALPHA (Laffort, Tortona (AL), Italy—strain A) and BIODIVA (Lallemand Inc., Montreal, QC, Canada—strain B) were used as starter yeasts. Both strains used as active dry yeast were rehydrated in water at 35 °C for 30 min and added to the apple juice within the next 30 min, conditioning the inoculum at a temperature not higher than 10 °C compared to the juice temperature. According to the manufacturer's instruction, 30 g/hL was the inoculum rate for strain A and 25 g/hL the one for strain B.

2.2. Experimental Design

Organic dessert apples of the Topaz cultivar (BioSüdtirol, Lana (BZ), Italy), from cold storage, were selected (discarding rotten fruit), rinsed with tap water, and crushed with a centrifugal mill (Voran®, Pichl bei Wels, Austria). The mash was pressed with a single-belt press (Voran®, Pichl bei Wels, Austria). No sulphite was added. The resulting cloudy juice was equally divided into 6 tanks, each filled with 25 L of juice. No correction of acidity was carried out because of the natural good balance of acid and sugars featuring Topaz

apple variety. Then, 25 g/hL of a complex yeast nutrient (Fermaid K, Lallemand Inc., Montreal, QC, Canada, supplying inorganic and organic nitrogen, vitamins, and minerals) was added for each tank. Each yeast strain was tested in triplicate (inoculum rate specified in Section 2.1). The fermentation was carried out at 20 °C and the sugar consumption was monitored daily in triplicate, with the aid of a pocket refractometer (PAL-BX/RI, Atago, Tokyo, Japan). In addition, the actual content of the residual glucose and fructose was verified through enzymatic assay described in the Materials and Methods section. Base ciders CA and CB represented the products obtained with strain A and B, respectively.

2.2.1. Pét-Nat Method (PN)

Once the residual sugar reached a glucose and fructose concentration of 22.6 g/L in tanks A and 26.4 g/L in tanks B, an aliquot of 2 L was taken from each respective tank, blended, and bottled (blend A (PNA) and blend B (PNB) from juice inoculated with strain A and B, respectively). Eight bottles for each blend were prepared. One bottle for each blend was equipped with a pressure gauge to monitor the fermentation follow-up.

2.2.2. Second Fermentation Methods

The remaining volume in each tank was left to ferment to dryness, blended (blend A and blend B prepared with fermented juice inoculated with strain A and B, respectively), and kept at 5 °C for 10–13 days for clarification before being racked off. Each blend was divided into two lots.

Bottle-fermented traditional or Champenoise (TM): One lot was added with 24 g/L sugar (in order to reach an overpressure inside the bottle of about 600 kPa), 25 g/hL of nutrient for yeast (Fermaid K, Lallemand Inc., Monteral, QC, Canada), and 15 g/hL of strain A or B, and bottled to ferment according to the Champenoise method. Prior to the sensory evaluation, the bottles were disgorged à la glace. The fermentations took place at room temperature and was monitored through pressure gauges. Final products will be indicated as TMA and TMB for strain A and B, respectively.

Tank-fermented Charmat method (CM): The second lot was used to fill pressure tank with 14.8 (in order to reach an overpressure of about 370 kPa in the tank) g/L sugar and 25 g/hL Fermaid K (Lallemand Inc., Montreal, QC, Canada), and inoculated with 15 g/hL of strain A and B according to the Charmat method. At the end of fermentation, the cider (CMA and CMB for strain A and B, respectively) was filtered and bottled under isobaric conditions. The fermentations took place at room temperature and was monitored through pressure gauges.

Experiment design is also shown in Figure S1.

2.3. Analytical Methods

2.3.1. Microbiological Analysis

The countable yeast population was verified in the apple juice before and after inoculation, at the bottling day for PN, at the end of base cider fermentation, and after inoculation for second fermentation in TM and CM. Plate count of serially diluted samples was performed on yeast peptone glucose (YPD) agar, used as reference, lysine agar to verify the absence of spontaneous *Saccharomyces* yeast overgrown in base cider), and Wallerstein Laboratory Nutrient Agar (WLNA) (to verify the absence of spontaneous apiculate overgrown in base cider). All plates were incubated at 25 °C for 3–4 days.

2.3.2. Physicochemical Parameters

The total soluble solid content was determined by measuring the refractive index of the solutions through a digital pocket refractometer (PAL-BX/RI, Atago, Tokyo, Japan). The pH was recorded using a portable pH meter equipped with 2Pore T electrode (XS Portable XS Instruments, Carpi (MO), Italy). Alcohol content and total acidity were analysed following the compendium of international methods of wine and must analysis [23].

2.3.3. Enzymatic Assays

Glucose, fructose and sucrose, malic acid, L-lactic acid, D-lactic acid, acetic acid, α-amino nitrogen, and ammoniacal nitrogen (the sum of these latter two measurements indicating the yeast assimilable nitrogen (YAN)), total polyphenols, catechin, and glycerol were measured by enzymatic analysis performed by semiautomatic analyser Italo S® (Exacta Optec, Verona, Italy) according to the manufacturer's instructions.

2.3.4. Chemical Analysis

Major volatile organic compounds (higher alcohols, esters, fatty acids, etc.) were analysed by headspace solid-phase micro-extraction with gas chromatography coupled with mass spectrometry (HS-SPME-GC-MS). A multipurpose sampler MPS robotic (Gerstel, Mülheim an der Ruhr, Germany) was used for HS-SPME injection. SPME extraction was carried out with a 1 cm SPME fibre with 65 µm of polydimethylsiloxane/divinylbenzene (Supelco) for 20 min (incubation temperature: 40 °C; incubation time: 10 min). A cooled injection system (CIS-4, Gerstel, Mülheim an der Ruhr, Germany) was used for transferring the sample to the gas chromatograph (GC 7890 A, Agilent, SC, USA). The method was fully described by Jung et al. [24]. The calibration was performed with two model wine solutions (3% and 6% v/v of ethanol, respectively), both containing 3 g/L of tartaric acid (pH 3), whereas 1-octanol was used for quantification and cumene as control standard. Aroma compound separation was performed using a 60 m × 0.25 mm × 1 µm gas chromatographic column (Rxi-5Sil, Restek, Bad Homburg, Germany). Detection was applied with a mass spectrometer MS 5975 B (Agilent, SC, USA) using EI (70 eV) and scan mode (m/z 35–250). Instrumental control, acquisition of data, and quantitative data analysis were performed using Agilent MassHunter workstation.

Headspace gas chromatography coupled with pulsed flame photometric detection (HS-GC-PFPD) was applied for the analysis of low-molecular-weight sulphur compounds. Details on sample preparation and instrument configuration can be taken from Jung et al. 2021 [24].

The analysis of monoterpenes, monoterpenoids, and C_{13}-norisoprenoids was carried out by headspace solid-phase micro-extraction in combination with gas chromatography and mass spectrometry (HS-SPME-GC-MS), according to the method described by Brandt [25].

2.4. Sensory Evaluation

Ciders were evaluated by a panel of 16 tasters. Samples were served at 10–12 °C in normalized black glasses (ISO 3591:1997 [26]). Four aroma attributes (floral, fruity, exotic, and vegetal), sweetness, acidity, bitterness, mouth intensity, and persistency of the effervescence and fault were the features rated on a continuous scale from 0 to 10. The tasting order was randomized. The ciders were poured all at the same time; one sample was given twice. If the difference between the rating of duplicated sample was bigger than the standard deviation of the whole panel for one attribute, the judge was not considered for this attribute.

2.5. Statistical Analysis

The physicochemical composition, the volatile organic compound (VOC) profile, and the sensory evaluation scores were statistically evaluated using a two-way ANOVA using Microsoft® Excel® for Microsoft 365 MSO (Version 2211 Build 16.0.15831.20098) 64-bit. Tukey's multiple-comparison post-hoc test was applied to determine the significant difference correlated to the following features: yeast strain, processing method, and their interaction. Principal component analysis (PCA) and heat map were realized using MetaboAnalyst 5.0 [27].

3. Results and Discussion

3.1. Microbiological Analysis

A microbial analysis was performed to verify the balance in the inoculum magnitude between the two strains. The count on the YPD medium has been considered as the reference. The initial microbial load in the juice was 2.15 $\log_{10}$ CFU/mL on YPD. The cell viability after the inoculum resulted in a count on YPD equal to 7.06 $\pm$ 0.05 $\log_{10}$ CFU/mL for strain A and of 7.26 $\pm$ 0.25 $\log_{10}$ CFU/mL for strain B. The cell population on the bottling day for the Pét-Nat cider was 7.60 $\pm$ 0.17 $\log_{10}$ and 7.36 $\pm$ 0.08 $\log_{10}$ CFU/mL for PNA and PNB, respectively, corresponding to 7 and 10 days of fermentation for CA and CB. After the second inoculum performed to trigger the fermentation in CMA, the enumeration counted 6.86 $\pm$ 0.13 $\log_{10}$ CFU/mL. In CMB, 6.76 $\pm$ 0.12 $\log_{10}$ CFU/mL were counted. In TM, 6.76 $\pm$ 0.09 $\log_{10}$ CFU/mL were counted for strain A, and 6.79 $\pm$ 0.01 $\log_{10}$ CFU/mL for strain B. For each method, the second fermentation, either in the bottle or pressurized tank, was started by a comparable cell density. In addition, few pieces of information have been collected for the base cider: at the end of the fermentation, the cell density for CA and strain CB was 7.53 $\pm$ 0.30 $\log_{10}$ CFU/mL and 6.53 $\pm$ 0.16 $\log_{10}$ CFU/mL, respectively, showing a population still quite stationary for strain A, reaching dryness (residual sugar below 1 g/L) after 11 days, and a population already in decline for strain B, reaching dryness after 14 days (residual sugar below 1 g/L). All cell counts reported above represent the colony-forming unit grown on the YPD medium. The count on Lysin agar had the same magnitude (data not shown), showing no proliferation during the base cider fermentation of the spontaneous *Saccharomyces* yeast. The count on the WLNA agar had the same magnitude as on YPD (data not shown) and all of the grown colony had a similar morphology, allowing the assumption of a homogeneous population.

3.2. Fermentation Monitoring

The concentration of sugars in the apple juice depends on the fruit variety, harvest time, storage, and climatic condition. Several physicochemical parameters were measured for the juice obtained from the apple cultivar Topaz (Table S1). The juice was characterized by 12 °Brix, 59.33 g/L sugar (sum of fructose and glucose) and 2.34 g/L of sucrose, pH 3.44, and a total acidity of 6.5 g/L. The YAN content was given as the sum of the α-amino nitrogen and ammoniacal nitrogen, both measured by enzymatic analysis. After the nutrient addition, a YAN concentration of 133 mg/L was estimated.

After the yeast inoculum, the performances of the two *T. delbrueckii* strains were assessed. The measurement of the sugar consumption showed that all fermentations had a regular kinetic, with the first fermentation being completed in 10 and 14 days by strain A and strain B, respectively (Figure S2). A similar fermentation trend was observed in apple juice fermented with *T. delbrueckii* by Fejzullahu et al. [28].

Sparkling ciders were produced according to three different methods: Pét-Nat (PN), the traditional bottle-fermented method (TM), and the tank-fermented Charmat method (CM). Both strains tested in our study carried out fermentation to dryness with no difference between methods, showing the ability to be active also under different overpressure. The similar observation for two *T. delbrueckii* strains utilized to produce sparkling wines have been described by Canonico et al. [29]. On the contrary, Ramirez et al. [21] and Velazquez et al. [30] observed a scarce tolerance to pressure higher than 350 kPa for other *T. delbrueckii* strains, suggesting that, also for this yeast species, the tolerance to high pressure is a strain-dependent factor.

For the PN cider, part of the fermenting products was bottled at a residual sugar content of 22.61 g/L, reached by strain A after 7 days, and 26.43 g/L, reached by strain B after 10 days. For each PNA and PNB bottle group, one manometer was mounted on one of the eight bottles to monitor the overpressure evolution during time. Both the PNA and PNB ciders showed a similar fermentation rate, reaching a constant pressure after 10 days This was considered as the fermentation end point for all the bottles.

To monitor the overpressure evolution, also for the TMA and TMB bottle group, one manometer was mounted on one bottle per type. In the TM ciders, according to the CO_2 development, the fermentation had a faster rate in the first 15–20 days, and a second slow phase, becoming constant within day 55 and day 56.

The CM cider inoculated with strain A showed a pressure of 4.5 bar after 51 days, while for strain B, 3.5 bar were measured after 57 days. In CM, the pressure was measured at the time the pressurized tank was opened for isobaric filtration and bottling, and, therefore, the time which included fermentation and maturation, established according to the usual CM method applied in many local cider production facilities.

3.3. Physicochemical Parameters

The physicochemical parameters of the final products were measured, and are reported in Table 1. The statistic difference among the samples and correlation to yeast strain, production method, or their interaction have been measured.

Most of the measured features appeared to be significantly influenced by the yeast strain and method interaction, with the exception of Brix, inorganic nitrogen content, catechins, and polyphenol content. The catechin content was significantly influenced by both the strains and the method, while the inorganic acids and the polyphenol content were significantly correlated to the methods.

Even if the ANOVA analysis revealed some significant difference of pH in the cider produced in this study, the most relevant difference was detected in the base cider produced by strain A (CA) and correlated to the higher total acidity measured for CA.

The total acidity was higher in CA (8.19 $\pm$ 0.08 g/L), compared to PNA, TMA, and CMA. On the contrary, the total acidity of CB was lower (4.82 $\pm$ 0.72 g/L) than in the related sparkling cider. Considering that the total acidity of juice was 6.5 g/L, it can be assumed that strain A has initially stimulated the synthesis of organic acids, lately consumed under CO_2 pressure. On the other hand, during the production of CB, organic acids were consumed and newly synthesized in the related sparkling wines. This behaviour may be associated with a strain-related response to different stress [31,32].

The differences in the detected acetic acid and malic acid were highly significant and correlated to the yeast strain, production method, and yeast strain–method interaction.

The higher amount of acetic acid was measured in ciders fermented with the *T. delbrueckii* strain B, and we also observed a lower concentration in the base ciders in comparison with the sparkling ciders. In particular, the base ciders CA and CB had a content about 2- and 3.6-fold lower than the related TM or CM.

The initial concentration of malic acid in juice (7.42 g/L) was higher than those recorded in ciders. The malic acid consumption was minimal in CA, with 6.71 $\pm$ 0.12 g/L left, and almost total in CB, with only 0.32 $\pm$ 0.45 g/L of malic acid left. In all sparkling ciders from both strain A and strain B, the malic acid was below the detection level. In some studies, it has been reported that *T. delbrueckii* is able to consume malic acid as a carbon source; this behaviour is strain-dependent, and, also, it depends on nutrient availability [33,34]. Together with malic acid consumption, the detection of L-lactic acid in all products, with the exception of CA, is suggesting the occurrence of malolactic fermentation. In addition, the presence of D-lactic acid, found in all products, can be a consequence of homo- and hetero-lactic acid bacteria metabolism [35].

The co-occurrence of acetic acid, L-, and D-lactic acid, and the little residual malic acid suggests the contemporary presence and active metabolism of the inoculate yeasts and spontaneous lactic acid bacteria already in the apple juice.

Table 1. Physicochemical characteristics of ciders produced with two different strains of *T. delbrueckii* and different fermentation methods aimed to produce sparkling cider.

	Significance			**Strain A**				**Strain B**			
	S	M	SxM	Base cider	Pet-Nat	Charmat	Champenoise	Base cider	Pét-Nat	Charmat	Champenoise
°Brix	ns	ns	ns	4.53 ± 0.05 ^{a}	4.33 ± 0.60 ^{b}	4.70 ± 0.08 ^{a}	4.47 ± 1.11 ^{a}	4.47 ± 0.05 ^{a}	4.80 ± 0.00 ^{a}	5.47 ± 0.66 ^{a}	5.33 ± 0.05 ^{a}
pH	***	***	***	3.42 ± 0.02 ^{a}	3.80 ± 0.00 ^{b}	3.80 ± 0.00 ^{b}	3.75 ± 0.03 ^{b}	3.85 ± 0.03 ^{b}	3.70 ± 0.03 ^{c}	3.76 ± 0.03 ^{bc}	3.73 ± 0.00 ^{bc}
Total acidity [g/L]	n	***	***	8.19 ± 0.08 ^{a}	4.45 ± 0.14 ^{b}	4.50 ± 0.05 ^{b}	5.24 ± 0.58 ^{c}	4.82 ± 0.72 ^{b}	6.20 ± 0.11 ^{c}	5.60 ± 0.48 ^{c}	6.16 ± 0.06 ^{c}
Glucose & Fructose [g/L]	***	***	***	0.02 ± 0.02 ^{a}	0.26 ± 0.16 ^{b}	0.09 ± 0.02 ^{b}	0.14 ± 0.01 ^{b}	0.66 ± 0.15 ^{c}	0.14 ± 0.04 ^{b}	0.04 ± 0.01 ^{a}	0.18 ± 0.03 ^{b}
Sucrose [g/L]	***	***	***	0.44 ± 0.03 ^{a}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.03 ± 0.04 ^{b}	0.03 ± 0.05 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}
Malic acid [g/L]	***	***	***	6.71 ± 0.12 ^{a}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.32 ± 0.45 ^{c}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}
D-lactic acid [g/L]	***	***	**	0.11 ± 0.00 ^{a}	3.47 ± 0.21 ^{b}	3.42 ± 0.13 ^{b}	4.22 ± 0.18 ^{c}	3.09 ± 0.47 ^{b}	5.02 ± 0.79 ^{c}	4.02 ± 0.23 ^{b}	5.11 ± 0.49 ^{c}
L-lactic acid [g/L]	Ns	***	***	0.00 ± 0.00 ^{a}	3.30 ± 0.16 ^{b}	4.21 ± 1.13 ^{b}	3.40 ± 0.11 ^{b}	2.75 ± 0.19 ^{c}	1.94 ± 0.12 ^{d}	2.70 ± 0.10 ^{c}	2.65 ± 0.16 ^{c}
Acetic acid [g/L]	***	***	***	0.16 ± 0.00 ^{a}	0.36 ± 0.10 ^{b}	0.37 ± 0.01 ^{b}	0.50 ± 0.01 ^{c}	0.44 ± 0.11 ^{c}	1.66 ± 0.15 ^{d}	1.49 ± 0.13 ^{d}	1.69 ± 0.15 ^{d}
α–amino nitrogen [mg/L]	*	***	**	3.33 ± 0.47 ^{a}	29.33 ± 2.2 ^{b}	17.33 ± 4.50 ^{c}	12.67 ± 2.87 ^{c}	3.33 ± 2.62 ^{a}	13.33 ± 2.62 ^{c}	16.00 ± 3.74 ^{c}	13.00 ± 1.63 ^{c}
Ammo- nium nitrogen [mg/L]	ns	***	Ns	17.00 ± 0.00 ^{a}	79.33 ± 2.87 ^{b}	83.67 ± 13.02 ^{b}	82.33 ± 20.75 ^{b}	22.33 ± 8.95 ^{a}	71.67 ± 20.33 ^{b}	72.33 ± 22.81 ^{b}	85.67 ± 30.92 ^{b}
Poly- phenols [mg/L]	ns	***	Ns	151.67 ± 23.81 ^{a}	27.00 ± 20.61 ^{b}	14.00 ± 10.42 ^{b}	10.00 ± 10.20 ^{b}	131.67 ± 51.98 ^{a}	2.00 ± 2.83 ^{c}	15.33 ± 18.93 ^{b}	16.33 ± 22.40 ^{b}
Catechins [mg/L]	*	**	Ns	41.00 ± 4.32 ^{a}	39.67 ± 1.25 ^{a}	25.67 ± 3.30 ^{b}	33.67 ± 6.02 ^{ab}	41.33 ± 3.09 ^{a}	22.33 ± 11.32 ^{ab}	24.00 ± 1.41 ^{b}	29.33 ± 1.70 ^{b}
Glycerol [g/L]	*	ns	*	5.56 ± 2.31 ^{ab}	4.65 ± 0.47 ^{a}	2.86 ± 2.06 ^{a}	4.65 ± 0.26 ^{a}	3.23 ± 0.09 ^{a}	6.48 ± 1.32 ^{ab}	5.62 ± 1.94 ^{ab}	9.89 ± 2.60 ^{b}
Ethyl Alcohol [%]	**	***	***	6.30 ± 0.08 ^{a}	6.35 ± 0.03 ^{a}	7.04 ± 0.03 ^{b}	7.37 ± 0.34 ^{b}	6.21 ± 0.16 ^{a}	6.00 ± 0.03 ^{c}	7.14 ± 0.32 ^{b}	7.43 ± 0.02 ^{b}

Data shown are average ± standard deviation of triplicates. The significance is indicated for strain (S), method (M), and the interaction of the two (SxM). ns: not significant; (***): significant at $p < 0.001$; (**): significant at $p < 0.01$; and (*): significant at $p < 0.05$. Different letters identify significant differences among samples.

In all final products, the residual sugar content was below 1 g/L; therefore, in all the products, the yeast fermented all the sugars available to dryness. Despite the ethyl alcohol content in all base ciders (6.30 ± 0.08 and 6.21 ± 0.16% for CA and CB, respectively) being very similar to the one of the corresponding PN ciders (6.35 ± 0.03 and 6.00 ± 0.03% for PNA and PNB, respectively), the ethyl alcohol concentration in CB was significantly different from PNB. Lorenzini et al. [11] and Fejzullahu et al. [28] reported a comparable ethanol production (5.2% vol) in apple juice inoculated with strain B although a residual content of 12.5 g/L and 12.1 g/L of glucose and fructose. The significantly higher amount of ethyl alcohol in the CM and TM ciders is related to the sugar addition to the base cider to trigger the bottle or tank fermentation and reach the overpressure of 370 and 600 kPa, respectively.

Glycerol is a by-product of the yeast sugar metabolism, from glucose to ethanol, during alcoholic fermentation. Glycerol enhances the mouthfeel, viscosity, and sweetness to the fermented beverage. Our analysis showed significant differences in glycerol production in ciders obtained from different strains, and related to the strain–method interaction: CA was characterized by a content in glycerol higher than CB, while the sparkling ciders obtained with strain B had a higher glycerol content than the respective ciders obtained with strain A. In a recent study on the NMR metabolic profiling of *T. delbrueckii* fermented apple juices, glycerol appeared to be among the more abundant metabolite [36]. In other studies, *T. delbrueckii* has been recognised for its impact in glycerol production in wine, from about 4.1 to 8.9 g/L, as reviewed by Ivit et al. [37]. In the same review, the increase in glycerol production from *T. delbrueckii*, related to anaerobiosis, was discussed. In this study, in bottle-fermented ciders, therefore with little or no dissolved oxygen, the amount of the measured glycerol was higher than in the corresponding open-tank-fermented cider CA and CB. In other fermented products, the glycerol production was related to the organic nitrogen availability [34].

The consumption of the α-amino nitrogen was significantly different in relation to the strain, production method, and strain–method interaction. In the base cider, TM, CM, and PN, the average residual organic nitrogen of 3.33 ± 1.88 mg/L, 12.83 ± 2.34 mg/L, 16.67 ± 4.12 mg/L, and 21.33 ± 8.46 mg/L, respectively, were measured. Similarly, the consumption of ammonium nitrogen was higher in the first fermentation than during in-bottle and autoclave fermentation. This can be explained by the higher yeast population density, the usual high rate of growth, the higher metabolic activity, together with the higher availability of oxygen, everything leading to a biomass increase, characteristic of the first fermentation in an open tank, compared to re-fermentation. In TM and CM, a higher residual nitrogen can be explained by the addition of a yeast nutrient to the base wine together with the yeast inoculum. The case is different for PN, in which no nitrogen supplement was added before bottling. However, PN was bottled before the end of the fermentation when the nitrogen was not totally depleted. Moreover, when preparing the blend from the original tank, a high amount of the solid was also taken from the partially fermented base cider tank. We can speculate that the residual nitrogen found at the end of the PN processing can result from the lack of consumption of nitrogen after the bottle was close and the presence in the bottle of the solid part for a long time. Differently from the base wine, in the PN bottle, the process was ended after a long time of contact with the solid and a higher surface-to-volume ration. In a previous study, cider fermented with a higher amount of the solid part resulted in a higher amount of residual nitrogen [38]. Further investigation is obviously necessary to better clarify this finding.

3.4. Polyphenols and Catechins

The total polyphenol content decreased with every fermentation step, showing significant differences between juice, base cider, and the final products, as shown in Table 1. Previous studies demonstrated the change in the content of phenolic compounds during the fermentation of apple juice [1]. Polyphenols may be condensed and precipitated during the fermentation and eliminated with the solids by disgorging (TM cider) or filtration (CM). In addition, the contact with oxygen during the process may have caused the decrease in

polyphenols as observed by Millet et al. [39], who found polyphenols in one haze sample, together with the markers of phenolic oxidation in another fraction of the French apple fermented product.

The fresh pressed apple juice had a significantly small amount of catechins (12.5 mg/L) compared to all fermented products (Table 1). Between the fermented products, there was a significant difference in the catechin amount: a maximum average value of 41.17 ± 3.76 mg/L was detected in the base ciders and a minimum average value of 24.83 ± 2.67 mg/L in the CM products. Our data support the observation of Minaar et al. [40], suggesting a positive contribution of *T. delbrueckii* in stimulating catechin release, therefore contributing to the product mouthfeel.

3.5. Terpenes and Norisoprenoids

A chemical analysis allowed the detection and quantification of six terpenes and norisoprenoids in juice, PN cider at bottling, base ciders, and base ciders after cold clarification as reported in Table 2. Terpineol, conferring citrusy and sweet aromas, was only detected in juice, while the other compounds were present in all the products with little differences associated with the strain, method, and the interaction of both. Among the other compounds, linalool, which can be enzymatically released from glycoside precursors, contributes to a flowery (rose) and fruity flavour (citrus) [41]. In CA and CB, linalool was detected at 2.32 (±0.01) and 1.78 (±0.02) µg/L, respectively. These concentrations were higher than those measured by Lorenzini et al. [11], suggesting that the Topaz apple cultivar contains higher amounts of glycosidic precursors. However, the linalool concentrations are very low and far below the odour thresholds [42]; therefore, no significant contribution to the overall aroma can be assumed. ß-damascenone, which contributes to a fruity aroma described as stewed apple and quince paste, was quantified in 1.87 (±0.01) and 1.55 (±0.01) µg/L for the ciders CA and CB, respectively. This amount is five times lower than the one detected in Golden Delicious ciders fermented by *T. delbrueckii* [11], suggesting a lower potential in the Topaz apple CV for β-damascenone release.

Table 2. Terpenes and norisoprenoids in ciders produced with two different strains of *T. delbrueckii* and different fermentation methods aimed to produce sparkling cider.

	Significance			Strain A				Strain B		
	S	**M**	**SxM**	**Juice**	**Cider**	**Pét-Nat**	**Cider AC**	**Cider**	**Pét-Nat**	**Cider AC**
Linalool oxide 1 (µg/L)	*	***	*	3.70 ± 0.22 ^{a}	4.41 ± 0.12 ^{a}	6.17 ± 0.55 ^{b}	4.11 ± 0.05 ^{a}	4.31 ± 0.02 ^{a}	5.17 ± 0.13 ^{c}	4.11 ± 0.15 ^{a}
Linalool (µg/L)	***	***	***	nd	2.32 ± 0.03 ^{a}	1.69 ± 0.03 ^{b}	2.09 ± 0.02 ^{c}	1.78 ± 0.02 ^{b}	1.39 ± 0.05 ^{d}	1.58 ± 0.0 ^{dc}
Hotrienol (µg/L)	ns	***	***	nd	9.14 ± 0.69 ^{b}	3.95 ± 0.08 ^{c}	3.77 ± 0.04 ^{c}	7.17 ± 0.30 ^{b}	3.02 ± 0.06 ^{d}	6.07 ± 0.16 ^{e}
α-Terpineol (µg/L)	ns	ns	ns	7.30 ± 0.03 ^{a}	nd	nd	nd	nd	nd	nd
Citronellol (µg/L)	ns	***	ns	nd	4.53 ± 0.73 ^{b}	nd	1.55 ± 0.01 ^{c}	3.77 ± 0.20 ^{b}	nd	1.29 ± 0.04 ^{d}
β-Damascenone (µg/L)	**	***	***	0.17 ± 0.01 ^{a}	1.87 ± 0.01 ^{b}	0.97 ± 0.14 ^{c}	1.55 ± 0.01 ^{d}	1.55 ± 0.01 ^{d}	1.18 ± 0.01 ^{c}	1.29 ± 0.04 ^{d}

Data shown are average ± std of triplicates. The significance is indicated for strain (S), method (M), and the interaction of the two (SxM). ns: not significant, (***): significant at $p < 0.001$; (**): significant at $p < 0.01$; and (*): significant at $p < 0.05$. nd: not detected. Different letters indicate significant differences among samples.

3.6. Volatile Sulphur Compounds

Volatile sulphur compounds may impact the perception of the fruity/floral attribute in cider because of their low aroma sensory threshold. No hydrogen sulphide, methanethiol, ethanethiol, thioacetic acid S-methyl ester, thioacetic acid S-ethyl ester, diethyl disulphide, and dimethyl trisulphide were detected in this study. Only carbon disulphide and dimethyl sulphide (DMS) were found in some of the ciders. Carbon disulphide was detected in

the TMB (2.8 ± 1.18 µg/L). DMS was found in a higher amount in the apple juice and in the sparkling ciders, with only traces found in the base ciders (Figure 1). Associated with an unwanted cooked note, DMS was detected in our study always below the odour threshold of 20–45 µg/L established in wine [43]. DMS is a sulphur compound that can be produced from methionine both by plants and by micro-organisms. Furthermore, DMS can be released from S-methylmethionine during the storage of wine [44]. It is present in apples, and it has been considered as a potential marker for flavour changes during the shelf life of apple juice, being sensitive to high temperature [44,45].

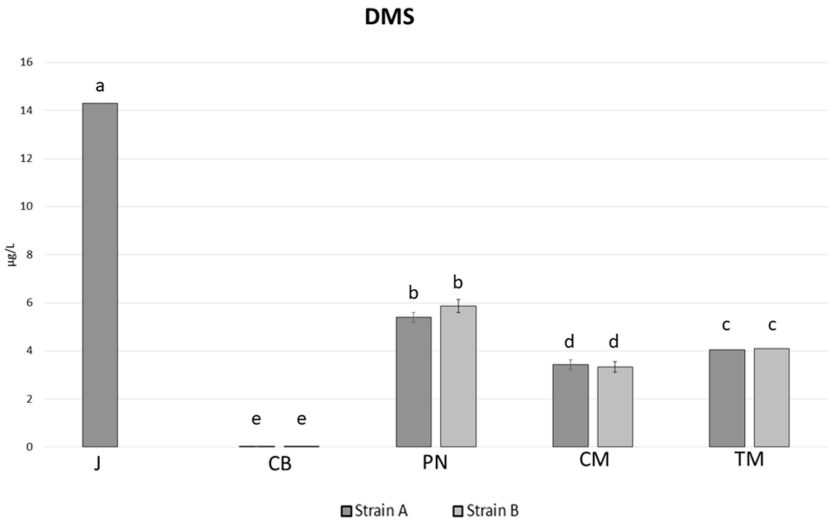


Figure 1. Dimethyl sulphide (DMS) amount found in apple juice (Juice), in base cider (Cider), Pét-Nat, pressured-tank Charmat method (Charmat), and traditional bottle-fermented cider (Champenoise) inoculated with *Torulaspora delbrueckii* strain A or strain B. Data are reported as average and error bars indicate the standard deviation. Different letters identify significant differences ($p < 0.05$).

3.7. Volatile Organic Compounds

Volatile compounds can be found in raw materials or may be formed during fermentation and maturation. The influence of *T. delbrueckii* on the volatile organic compound (VOC) production in cider has been scarcely investigated, while more information is available for wine and beer [19,46], where *T. delbrueckiii* has been associated with an enhanced fusel alcohol and ester production.

Esters, alcohols, and acids are the main constituents of the fermentation bouquet [21]. Twenty-six VOCs were detected and quantified (examples of the chromatograms are reported in Figure S3a,b), whereby 17 were identified as esters (11 ethyl esters), five as alcohols, and four as acids. Most of the VOCs appear to be produced during fermentation as previously observed [47] and only the 2-methylbutyric acid methyl ester was exclusively found in the juice sample.

To evaluate the correlations among the VOCs, strains, and production method, a principal component analysis (PCA) was carried out. The PCA-score plot and biplot (Figure 2) are showing the separation of ciders fermented with strain A from samples fermented with strain B (Figure 2A) according to their metabolite profile. PC1 explained 32.3% of the total variance and allows us to identify the compounds correlated to yeast strains, while PC2, explaining 28.2% of the total variance, allows a separation of the samples based on the production methods (Figure 2B). The ciders obtained with strain A clustered in the right side of the score plot, correlating with alcohols and most of the detected esters. The ciders fermented by strain B clustered on the left side of the plot correlating with acids and some esters (Figure 2B). For both strains, the base ciders were placed on the upper side of the plot, while the sparkling ciders were also influenced by negative loadings and partially or totally projected on the lower part of the plot.

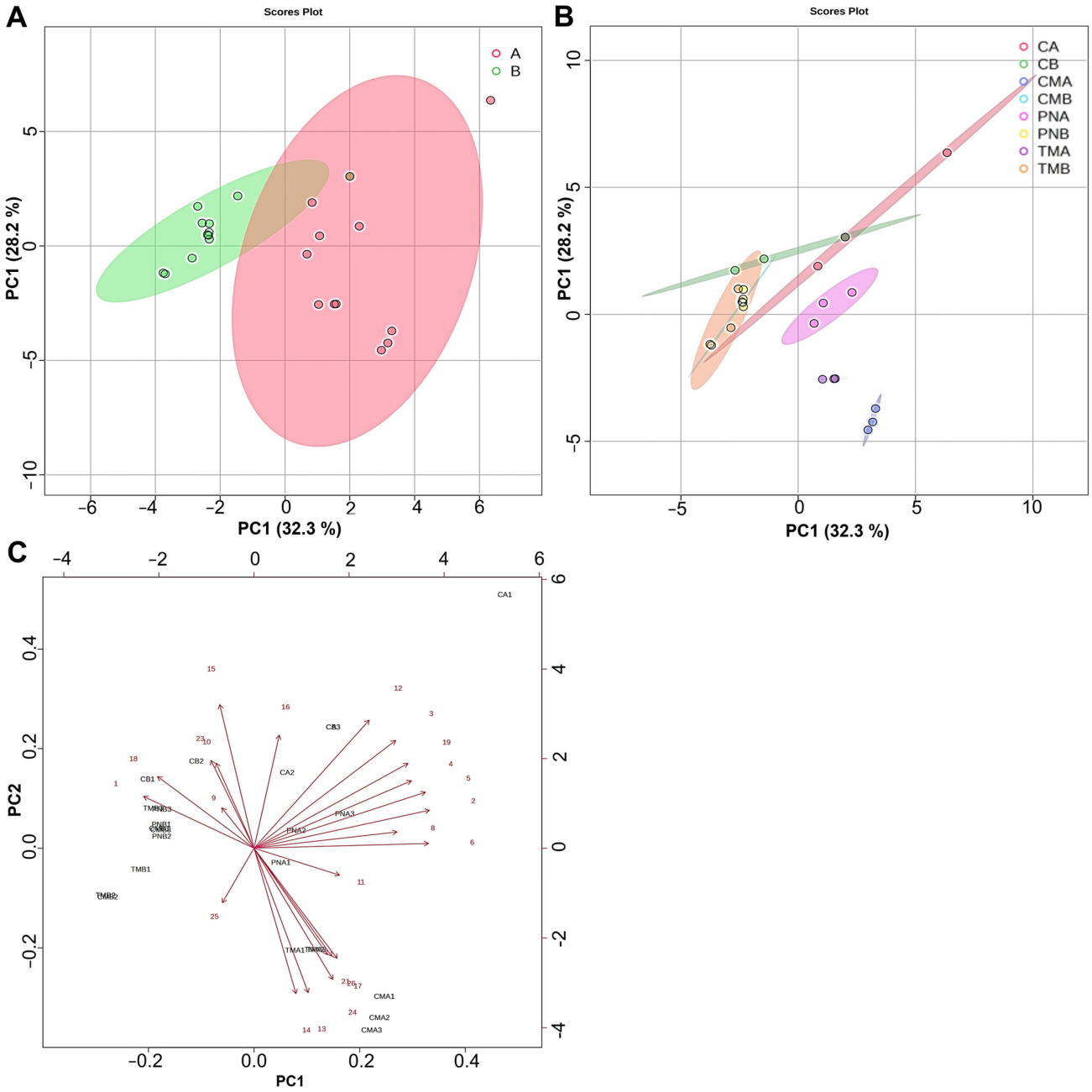


Figure 2. Principal component analysis of volatile organic compounds: (**A**) Score plot of ciders produced with different *T. delbrueckii* strains; (**B**) score plot of ciders obtained with different methods; (**C**) biplot based on VOC composition of samples. C: base cider; PN: Pét-Nat; TM: Champenoise method, CM: Charmat method. A and B indicate ciders fermented by T. delbrueckii strain A and strain B, respectively. (1) Acetic acid ethylester; (2) i-butanol; (3) propionic acid ethylester; (4) 3-methyl-butanol; (5) 2-methyl-butanol; (6) i-butyric acid ethylester; (7) 2-methylbutyric acid methylester; (8) butyric acid ethylester; (9) lactic acid ethylester; (10) i-valeric acid; (11) 2-methylbutyric acid ethylester; (12) hexanol; (13) acetic acid 3-methylbutylester; (14) acetic acid 2-methylbutylester; (15) hexanoic acid; (16) hexanoic acid ethylester; (17) acetic acid hexylester; (18) 2-hydroxy-4-methylvaleric acid ethylester; (19) 2-phenyl-ethanol; (20) octanoic acid; (21) succinic acid diethylester; (22) octanoic acid ethylester; (23) benzeneacetic acid ethylester; (24) acetic acid phenylethylester; (25) decanoic acid; and (26) decanoic acid ethylester.

The final products obtained with strain B have been separated in a cluster showing the least diverse volatile composition. These results are better highlighted in the heatmap (Figure 3) where the distance between the links is smaller among the strain B cider clusters than strain A cider clusters.

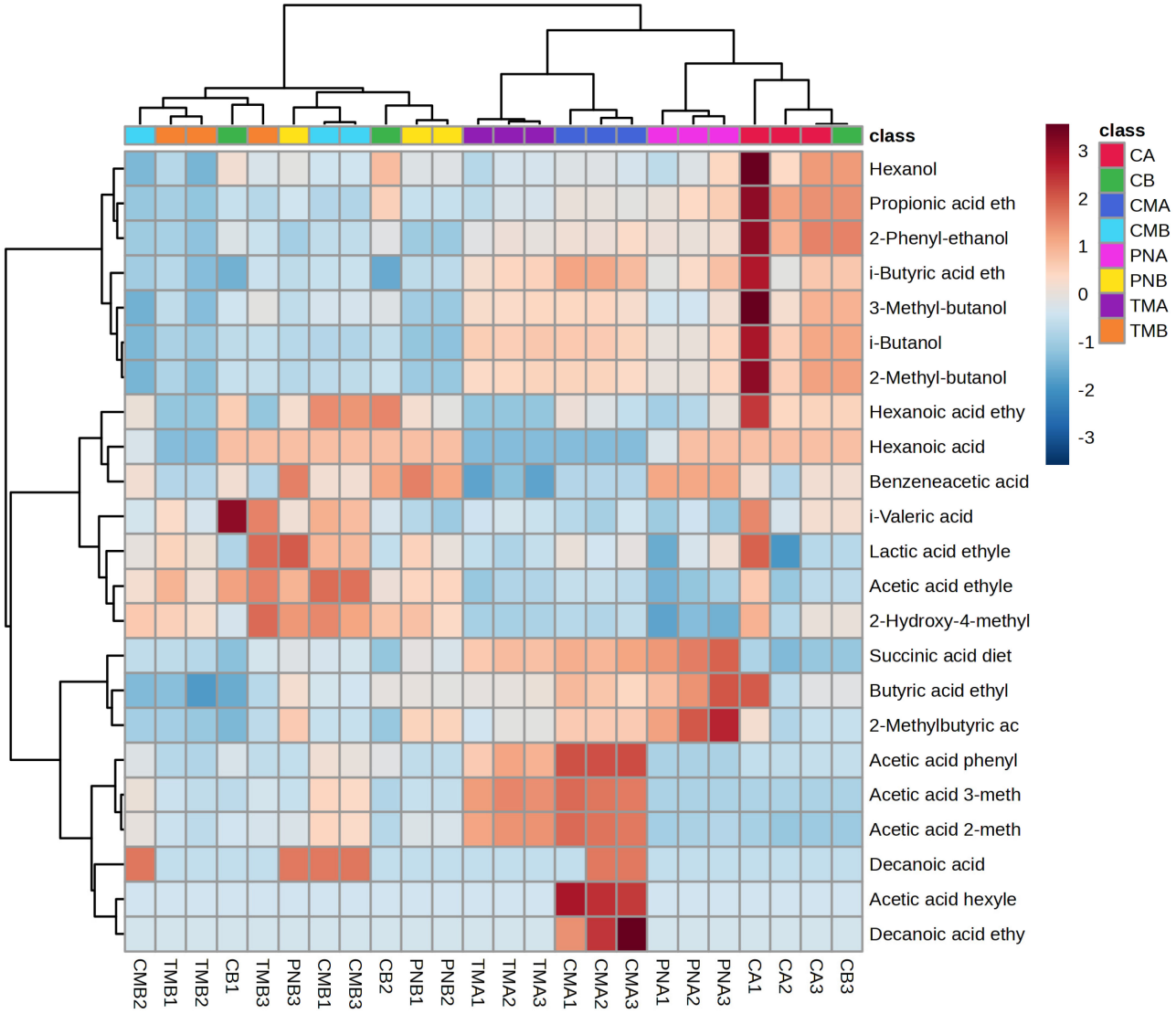


Figure 3. Heat map representing the cider volatile organic compound profile: the darker the colour, the higher the concentration of the compound in the sample. The 6 replicates of each sample are represented. Class A and B are representing the samples of cider produced by *T. delbrueckii* strain A strain B, respectively. C: base cider; PN: Pét-Nat; TM: Champenoise method, CM: Charmat method. A and B indicate ciders fermented by *T. delbrueckii* strain A and B, respectively.

The quantification of the VOCs is reported in Table S2a,b. The ANOVA analysis shows significant differences among the strains for all the metabolites, while the applied production method generated significant differences for 20 compounds. The sample produced with the Champenoise method contained more decanoic acid, decanoic acid ethyl ester, and acetic acid hexyl ester than the other samples. An interaction between the strain and method was noted for 14 of the VOCs, namely, for acetic acid ethyl ester, i-butanol, propionic acid ethyl ester, 2-mehtylbutanol, i-butyric acid ethyl ester, acetic acid 3-methylbutylester, acetic acid 2-methyl butyl ester, hexanoic acid ethyl ester, acetic acid hexyl ester, 2-hydroxy4-methylvaleric acid ethyl ester, 2-phenyl-ethanol, succinic acid diethyl ester, benzene acetic acid ethyl ester, acetic acid phenyl ethyl ester, and decanoic acid ethyl ester.

3.7.1. Higher Alcohols

Higher alcohols, which contribute to a warm mouth feeling, are produced during the fermentation from sugars and amino acids via the Ehrlich metabolic pathway, as reviewed in Hazelwood et al. [48]. Quantitatively, the higher alcohols represented the largest group (Figure 2), accounting for the 66% of the VOCs. The total amount of higher alcohols in the ciders showed highly significant differences due to the strain and production methods (Table S3). In the cider produced with strain A, the concentration of volatile alcohols significantly higher than in ciders from strain B were found (Figure 4). In particular, the alcohol content in the CA, TMA, and CMA ciders was similar, but higher than the other ciders.

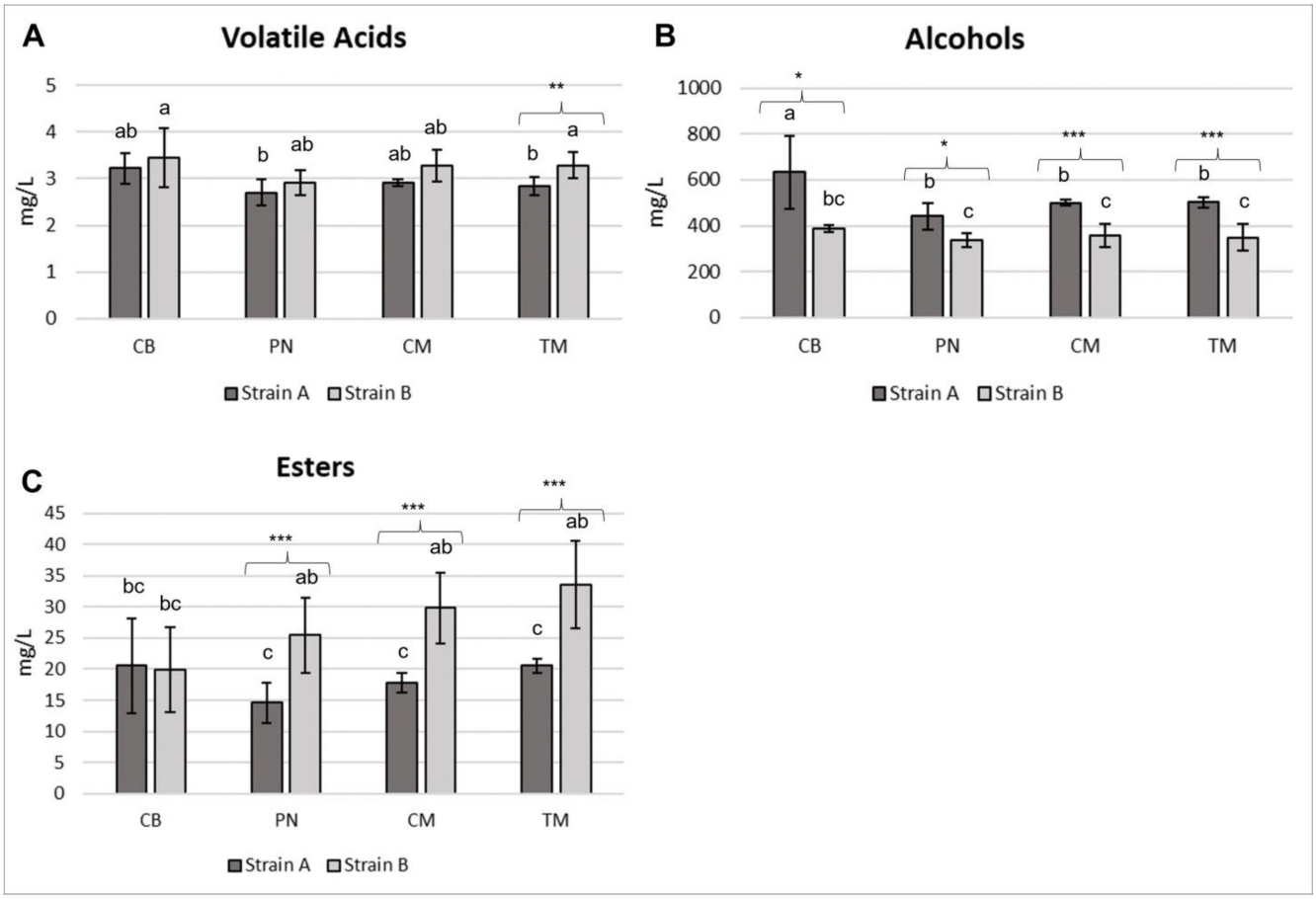


Figure 4. Sum of volatile (**A**) alcohols, (**B**) acids, and (**C**) esters measured in base cider (Cider), Pét-Nat, pressured-tank Charmat method (Charmat), and traditional bottle-fermented cider (Champenoise) inoculated with *Torulaspora delbrueckii* strain A or strain B. Data are reported as average and error bars indicate the standard deviation. Differences between strains for method are indicated as (***): significant at $p < 0.001$, (**): significant at $p < 0.01$, and (*): significant at $p < 0.05$. Different letters identify significant differences ($p < 0.05$).

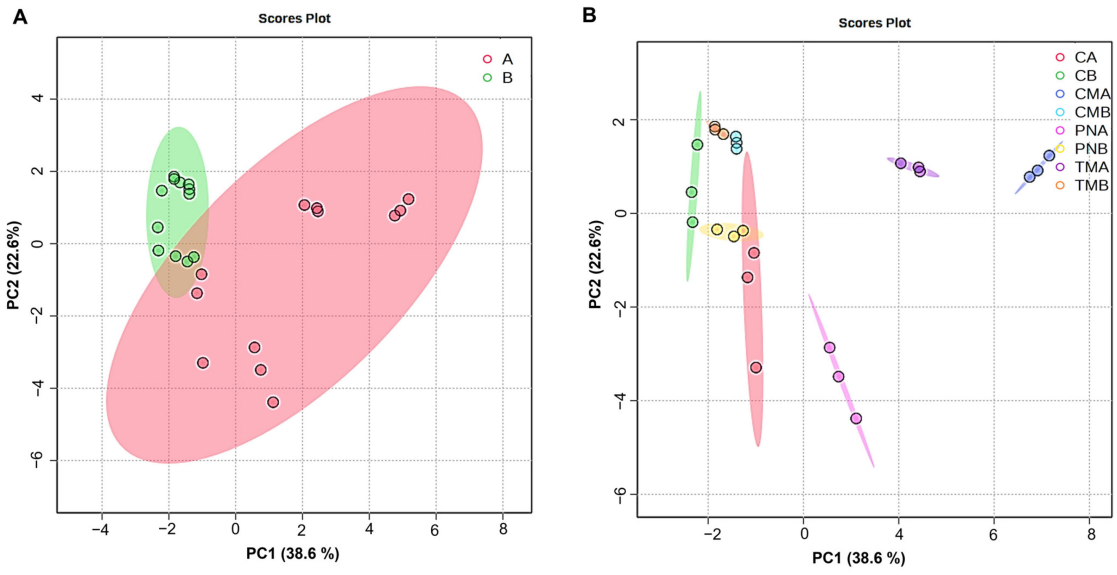
i-Butanol, 2-methylbutanol, 3-methylbutanol, hexanol, and 2-phenylethanol were detected in all the samples: hexanol, whose odour is associated with herbaceous, green, and sweet notes, was the major quantified alcohol in all the obtained products, followed by 3-methyl-butanol, associated with a fruity banana aroma [1]; comparable quantities of hexanol and i-butanol were detected by Lorenzini et al. [11] when fermenting apple juice with *T. delbreuckii;* 2-phenyl-ethanol, associated with a rose- and honey-like aroma, has been previously detected in ciders [28].

3.7.2. Volatile Acids

Concerning the total amounts of volatile acids, significant differences ($p < 0.05$) have been linked to the strain and production method (Figure 4). Decanoic acid was only detected in ciders produced with the Champenoise method and in minor amounts in PNB (Table S2a,b). TMB had significantly higher amounts of acids in comparison to TMA. The amount of hexanoic and octanoic acid produced by the two strains of *T. delbrueckii* was below the sensory threshold [47], leaving i-valeric acid to be the only volatile carboxylic acid above the flavour threshold of 0.3 µg/L [49] (Table S2a,b). i-Valeric acid, associated with a milky taste, could, to some extent, contribute to the aroma profile of ciders, conferring a delicate fruity floral profile.

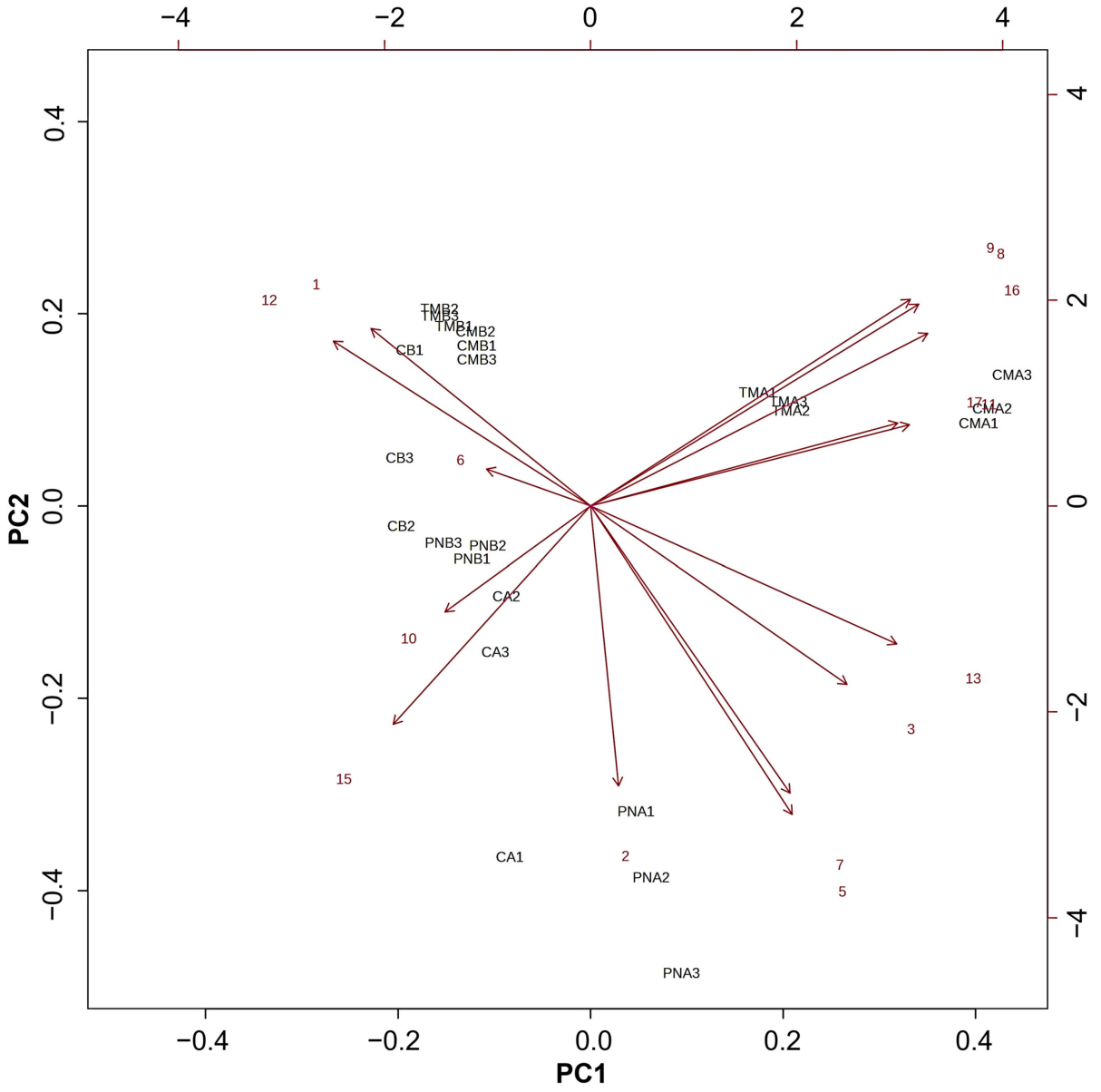
3.7.3. Esters

Esters are fermentative metabolites produced by yeasts via the enzymatic reaction involving a carboxylic acid and an alcohol (Figure 4). They represent key flavour compounds in fermented beverages, contributing to a pleasant fruity aroma. *T. delbrueckii* has a deep influence on the development and production of esters in wine [20]. In our study, esters accounted for the 33% of the quantified compounds. A PCA analysis of the esters (Figure 5) allowed a separation of the samples according to PC1 and PC2, explaining the 37% and the 22.3% of variance, respectively. PC1 contributes to separate samples in relation to the yeast strain employed for the fermentation. The ciders obtained with strain B formed a small cluster on the left side of the graph, correlating with the lactic acid ethyl ester, acetic acid ethyl ester, and 2-hydroxy-4-valeric acid ethyl ester. Among the strain A ciders, CA is the only product located on the left and lower side of the graphic together with PNB, and their position is correlated with hexanoic acid ethyl esters and with benzene acetic acid ethyl ester as negative loadings. Sparkling ciders produced with strain A are located on the right side of the graphic with a separation along PC2 among PNA, on the lower side of the graphic, and samples obtained with the Charmat and Champenoise methods that are positioned in the upper right quadrant. PNA is closer to CA than CMA and TMA, and correlated with the esters of butyric acid, succinic acid diethyl ester, and propionic acid diethyl ester. The CMA and TMA ciders correlated with acetic acid 2-methylbutylester, acetic acid 3-methylbutylester, acetic acid phenyl ethyl ester, decanoic acid ethyl ester, and acetic acid ethyl ester.



(**a**)

Figure 5. *Cont.*



(**b**)

Figure 5. (**a**). PCA analysis of esters. (**A**): Score plot of ciders produced with different *T. delbrueckii* strains. PC1 accounts for 37% of the variability; PC2 accounts for 22.3% of the variability. (**B**): Score plot of ciders obtained with different methods: base cider (C), Pét-Nat (PN), Champenoise method (TM), Charmat method (CM). In sample label, letter A and B indicate ciders fermented by *T. delbrueckii* strain (A,B), respectively. (**b**). PCA analysis: biplot based on chemical analysis of esters: base cider (C), Pét-Nat (PN), Champenoise method (TM), Charmat method (CM). In sample label, letter A and B indicate ciders fermented by *T. delbrueckii* strain A and B, respectively. (1) Acetic acid ethylester; (2) propionic acid ethylester; (3) i-butyric acid ethylester; (4) 2-methylbutyric acid methylester; (5) butyric acid ethylester; (6) lactic acid ethylester; (7) 2-methylbutyric acid ethylester; (8) acetic acid 3-methylbutylester; (9) acetic acid 2-methylbutylester; (10) hexanoic acid ethylester; (11) acetic acid hexylester; (12) 2-hydroxy-4-methylvaleric acid ethylester; (13) succinic acid diethylester; (14) octanoic acid ethylester; (15) benzeneacetic acid ethylester; (16) acetic acid phenylethylester; and (17) decanoic acid ethylester.

For all the identified esters, an ANOVA analysis was performed and significant differences in the compound concentration due to the strain, method, and method–strain interactions were highlighted (Table S2a,b).

Acetic acid ethyl ester is the most common ester produced during alcoholic fermentation. It can be produced by both fermentative yeasts and acetic acid bacteria in low-oxygen conditions. In all the analysed samples, acetic acid ethyl ester was the most abundant ester, ranging from 11.36 mg/L to 31.11 mg/L in PNA and TMB, respectively. Acetic acid ethyl ester, when in a low concentration, is associated with a fruity aroma, while, when its concentration exceeds the human perception threshold (100–200 mg/L), it is associated with an unpleasant aroma of solvent [50].

Succinic acid diethyl ester, associated with a stewed fruit aroma, and i-butyric acid ethyl ester, with a fruity, pineapple, and floral aroma, were produced in a concentration two times higher in PNA, CMA, and TMA ciders in comparison with the respective sparkling ciders produced with the *T. delbreuckii* strain B.

The i-butyric acid ethyl esters were detected in a higher amount compared with other ciders [11]. The abovementioned esters were described among the most active aroma compounds of North American and Chinese ciders together with the 2-methylbutyric acid ethyl ester (fruity) and decanoic acid ethyl ester (grape) [51].

It is worth noting that the total amount of esters in sparkling ciders produced with strain B was significantly higher than those detected in sparkling ciders obtained with strain A (Figure 4). As mentioned above, the synthesis of esters depends on the alcohol and organic acid availability. In this study, a negative correlation between the esters and higher alcohols was detected in sparkling ciders obtained with different yeast strains. It is possible to hypothesize that the alcohols in the strain B ciders have been driven to a higher ester synthesis, justifying the lower volatile alcohol content of these ciders.

Another factor influencing ester biosynthesis could be the increasing pressure of carbon dioxide during fermentation. In our study, we found a decrease of the concentration of some esters in sparkling ciders (e.g., hexanoic acid ethylester), while other esters were produced in higher amounts (e.g., acetic acid 3-methylbutylester, acetic acid 2-methylbutylester, succinic acid diethylester, and acetic acid phenyl ethyl ester). Acetic acid hexyl ester and decanoic acid ethylester were only detected in ciders from the Champenoise method and strain A. Despite the fact that, in wine, it has been observed that a light overpressure led to an increase in ester production during fermentation [52], other studies report that high pressures negatively affected their biosynthesis [53]. Thus, the significantly lower amounts of esters in sparkling ciders obtained with strain A, in comparison with strain B, could be, to some extent, influenced by the higher CO_2 pressure developed during the production of PNA, TMA, and CMA.

3.8. Sensory Analysis

A sensory evaluation was carried out by a semi-trained panel of 16 cider experts. All ciders have been mainly described as fruity and acidic. None of the sensory attributes was significantly higher in the tested samples (Figure 6); the samples produced with strain B obtained the higher score for the exotic, acidity, bitterness, and effervescence (*perlage)* intensity and persistency attributes. The PN ciders obtained higher scores for the floral, exotic, sweetness, and fault intensity attributes. The CM ciders were described as more sparkling, bitter, and with more persistent bubbles in the mouth. The cider produced according to the TM method scored high points for the fruity, vegetal, and acidity attributes. On a scale from 0 to 10, the strain B samples were described with an off-flavour intensity of 1.30 $\pm$ 0.17, slightly significantly higher than the strain A samples (0.49 $\pm$ 0.33). The off-odour was also described by some judges as a sweaty odour correlated to the i-valeric acid [48], quantified in these samples to be above the threshold.

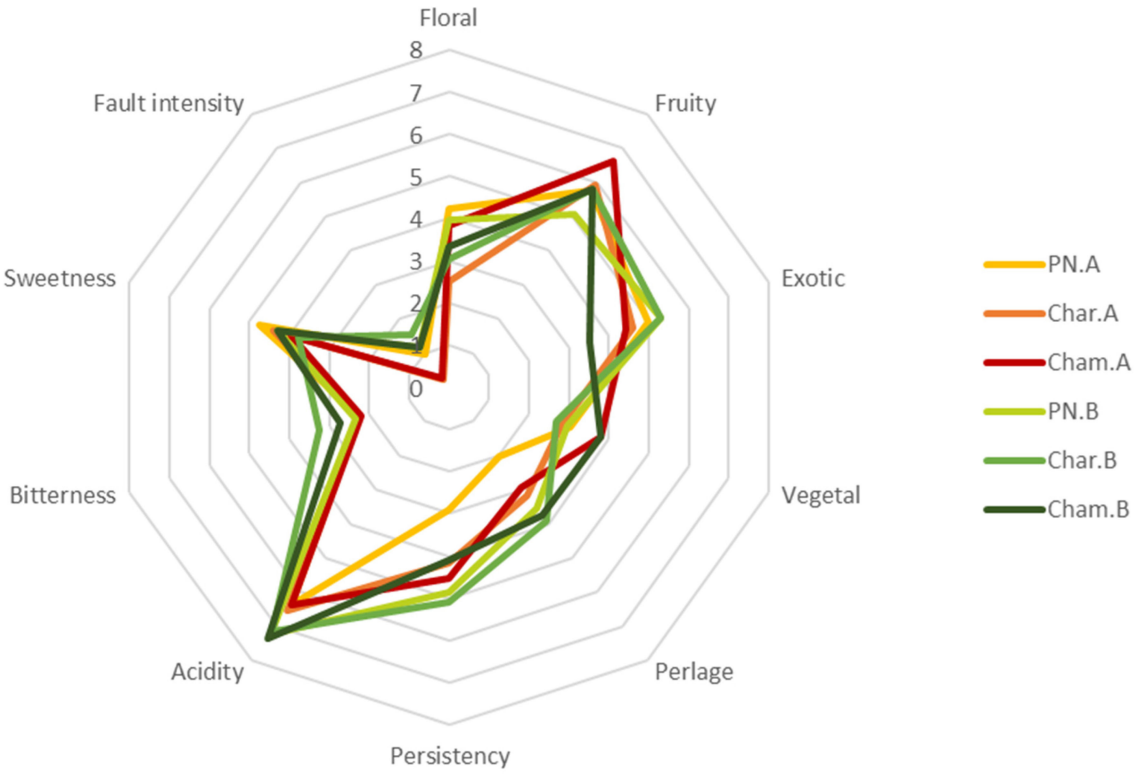


Figure 6. Sensory analysis aroma profile of the final products. PN = Pét-Nat, Char = Charmat method, and Cham = Champenoise method in cider produced after inoculum with *Torulaspora delbrueckii* strain A (A) or strain B (B). The attributes were assessed on a continuous scale from 0 to 10, with 0 being the lowest and 10 the highest score. Perlage: effervescence intensity; persistency: effervescence persistency.

4. Conclusions

In the search for new actors able to confer unique aromatic characteristic to ciders, we tested the ability of two commercially available *T. delbrueckii* strains to ferment apple juice under different conditions. The obtained results show that both *T. delbrueckii* strains were able to withstand high pressure and deplete all the sugars, producing a correlated amount of ethyl alcohol. The profile of volatile organic components for each yeast strain was documented as well as their capability to produce different amounts of glycerol and organic acids, offering to the cider maker the possibility to master the extract and mouthfeel using selected yeasts. Despite the differences detected in volatile organic compounds, both strains imparted sensorial characteristics dominated by a fruity/floral aroma. In conclusion, our study contributes to expand the knowledge on *T. delbrueckii* behaviour during apple juice fermentation in relation to volatile and non-volatile metabolite production and paves the way for using *T. delbrueckii* as a solo player to produce ciders with unique aroma profiles.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13064015/s1, Figure S1: Experiment design and flowchart: C: base cider; PN: Pét-Nat method; CM: tank-conditioned or Charmat method; TM: bottle-conditioned or Champenoise method; A: strain A; and B: strain B; Figure S2: Fermentation monitoring shows the fermentation curve for base cider expressed as sugar consumption measured by refractometer (Brix). The recorded values have been adjusted for ethanol effect; Figure S3: (a) Volatile compounds measured by HS-SPME-GC-MS-analysis in traditional bottle-fermented cider (Champenoise) inoculated with *Torulaspora delbrueckii* strain A; (b) Volatile compounds measured by HS-SPME-GC-MS-analysis in traditional bottle-fermented cider (Champenoise) inoculated with *Torulaspora delbrueckii* strain B; Table S1: Physiochemical composition of Topaz apple juice with added yeast nutrient prior to inoculation; Table S2: (a) Volatile organic compounds in ciders produced with

strain A also compared to the apple juice; (b) Volatile organic compounds in ciders produced with strain B also compared to the apple juice; Table S3: Organic volatile compounds in ciders produced with two different strains of *T. delbrueckii* and different methods to produce sparkling cider.

Author Contributions: Conceptualization, S.S., D.R. and L.C.; data curation, N.T.; formal analysis, M.E., P.H., C.S., B.B., S.B. and S.F.; funding acquisition, L.C.; investigation, M.E., D.R. and L.C.; methodology, M.E., D.R. and L.C.; project administration, L.C.; resources, D.R. and L.C.; supervision, S.S., D.R. and L.C.; validation, D.R.; writing—original draft, M.E.; writing—review & editing, N.T., S.S., D.R. and L.C. All authors have read and agreed to the published version of the manuscript.

Funding: Laimburg Research Centre is funded by the Autonomous Province of Bozen-Bolzano. The Autonomous Province of Bolzano, Department of Innovation, Research and University is gratefully acknowledged for its financial support for the Capacity building 1 (Decision n. 1472, 07 October 2013) and the project Step-Up (Decision n. 864, 04 September 2018).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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