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Mixed Fermentations of Yeasts and Lactic Acid Bacteria as Sustainable Processes to Enhance the Chemical Composition of Cider Made of Topaz and Red Topaz Apple Varieties

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Abstract: This study examined the effect of simultaneous fermentations of *Saccharomyces cerevisiae* and *Pichia kluyveri*, *Lactobacillus plantarum* and *Oenococcus oeni* on the chemical composition of apple cider from two apple varieties—Topaz and Red Topaz. Analytical techniques (HPLC-RID, HPLC-VWD, GC/MS, GC/FID, HPLC-DAD ESI+) were employed to analyze glucides, organic acids, volatile compounds, amino acids and phenolic compounds, respectively. Statistical analysis and and PCA were conducted to assess the correlations among samples based on the compounds identified. In the mixed fermentations, such as *Saccharomyces cerevisiae* + *Lactobacillus plantarum* and *Saccharomyces cerevisiae* + *Oenococcus oeni*, the amount of lactic acid was higher compared to the other samples, thus proving the effectiveness of malolactic fermentation simultaneous to alcoholic fermentation. The fermentation of *Saccharomyces cerevisiae* + *Pichia kluyveri* resulted in the formation of greater amounts of certain volatile compounds. Moreover, the sensory analysis revealed that *Saccharomyces cerevisiae* + *Pichia kluyveri* distinguished apple-like, fruity and floral notes. This study suggests that the simultaneous inoculation of *Saccharomyces* and non-*Saccharomyces* yeasts results in a more complex-flavored cider. The mixed fermentation of yeast and lactic acid bacteria is a sustainable method given the shortened fermentation duration and can be successfully applied in the cider industry.

Keywords: apple cider; simultaneous fermentation; sustainability; *Pichia kluyveri*; *Saccharomyces*; lactic acid bacteria; composition

1. Introduction

Cider is an alcoholic beverage produced from apple juice that has gone through alcoholic fermentation (AF) with yeasts and, frequently, malolactic fermentation (MLF) with lactic acid bacteria (LABs) [1]. These yeasts and bacteria play a crucial role in the fermentation of fruit wines by producing flavoring substances, such as esters, organic acids and terpenes [2]. On the one hand, yeasts, primarily represented by *Saccharomyces cerevisiae*, are responsible for AF that leads to the formation of ethanol, esters, higher alcohols and other important volatile compounds [3]. Several non-*Saccharomyces* yeasts are increasingly being utilized, especially with wine and cider, mostly because they can improve the fermented beverages' flavor complexity and sensory attributes [4,5]. *P. kluyveri*, the only *Pichia* species commercially available on the market, is also the most thoroughly

studied of the *Pichia* species and is recognized for its ability to enhance the aromatic profile of wines, especially with increased contents in fruity esters, terpenes and thiols [6].

MLF is a secondary biological fermentation that begins just after AF and is primarily elicited by LABs, such *Lactobacillus*, *Oenococcus*, *Pediococcus* and *Leuconostoc* [7]. Still, MLF is not compulsory in cider processing. It is a common operation in English and French cider processing; however, in the United States, where craft cider it beginning to become extremely popular, it is often marketed as young without passing through MLF [2]. But, to achieve lower acidity, eliminate residual nutrients, ensure microbial stability and create complex aromatic profiles, lactic acid bacteria (LABs) may be used in MLF [8]. During the spontaneous MLF of cider, *Lactobacillus* and *Oenococcus* are described as the predominant species, but *Leuconostoc* and *Pediococcus* are commonly found in a lower amount [9]. As a result of MLF, L-malic acid is converted into L-lactic acid, a beneficial process that enhances the cider's sensory traits by lowering its acidity and simultaneously increasing its microbiological stability [10]. Because of its tolerance to the challenging fermentation conditions (low pH, high alcohol and SO_2) and the fact that it typically produces lower quantities of biogenic amine metabolites, *O. oeni* is the most effective LAB for MLF [8,11]. On the contrary, *Lactobacillus* has received a lot of attention related to malolactic fermentation because it may produce greater flavor-related enzymes than *O. oeni*. Furthermore, multiple studies have shown that *Lactobacillus* strains can be used in yeast mixtures to improve the quality of fruit-based fermented beverages [12]. In general, MLF takes place after AF, and MLF bacteria are spontaneously or sequentially inoculated following AF to prevent the increased content of acetic acid [8]. However, the production of alcohol and yeast metabolites, such as fatty acids and SO_2, during AF might negatively influence the growth of some *O. oeni* strains, which delays MLF [13,14]. Moreover, apples and pears have lower nutrient contents compared to grapes [11], which yeasts quickly and almost entirely consume during AF. In general, the higher the ethanol content, the longer it takes for LABs to adapt and grow and for MLF to occur [15]. LABs need nutrients, including a small amount of sugar, amino acids, vitamins and minerals, to grow. After the alcoholic fermentation and yeasts autolysis, they become a source of economic nutrients for LABs [16].

Like for the wine industry [17], cider fermentation is the most time- and energy-consuming operation. Therefore, there is a clear need for cider industrials to reduce the entire fermentation process while maintaining the product quality.

All of the abovementioned issues can be resolved by the simultaneous inoculation of yeast and bacteria, which permits the bacteria to easily adapt to the challenging fermentation environment and, thus, shorten the entire fermentation process. This procedure was reported previously on for wine [12,18] and cider [19], but, to the best of our knowledge, this is the first time it has been adapted for cider fermentation using these yeasts and LAB strains. This study aimed to explore the effect of cofermentation with *Saccharomyces* + *Pichia* and *Saccharomyces* + LAB, respectively in the cider processing of two Romanian apple varieties. The cider variants were assessed in terms of sugars, organic acids, volatile and phenolic compounds, and amino acids, as well as underwent sensory analysis.

2. Materials and Methods

2.1. Materials and Experimental Design

Pasteurized apple juice (AJ) was obtained from Topaz and Red Topaz apple varieties (ratio 1:1) harvested in September 2022. AJ was delivered by a local orchard located near the city of Cluj-Napoca (Central Romania), at 46°48′21.4″ N 23°35′19.6″ E. Using a Consort C532 pH-meter (Consort, Brussels, Belgium) and a portable refractometer DR 201-95 (Kruss, Hamburg, Germany), respectively, the pH and Brix degrees were measured before and during fermentation.

The apple juice was subjected to fermentation processes to obtain cider (Figure 1). Four yeast strains were selected for cider production: *P. kluyveri* (Viniflora® FrootZen™, Chr. Hansen, Hoersholm, Denmark) 10 g/hL, *S. cerevisiae* (Viniflora® JAZZ™, Chr.

Hansen, Hoersholm, Denmark) 25 g/hL, *O. oeni* (Viniflora® SPARTA™, Chr. Hansen, Hoersholm, Denmark) 25 g/hL and *L. plantarum* (Viniflora® NoVa™, Chr. Hansen, Hoersholm, Denmark) 17 g/hL. The microorganism inoculations were in accordance with each strain manufacturer. This resulted in 4 cider variants: *P. kluyveri + S. cerevisiae* (C1), *L. plantarum + S. cerevisiae* (C2), *O. oeni + S. cerevisiae* (C3) and *S. cerevisiae* (C4).
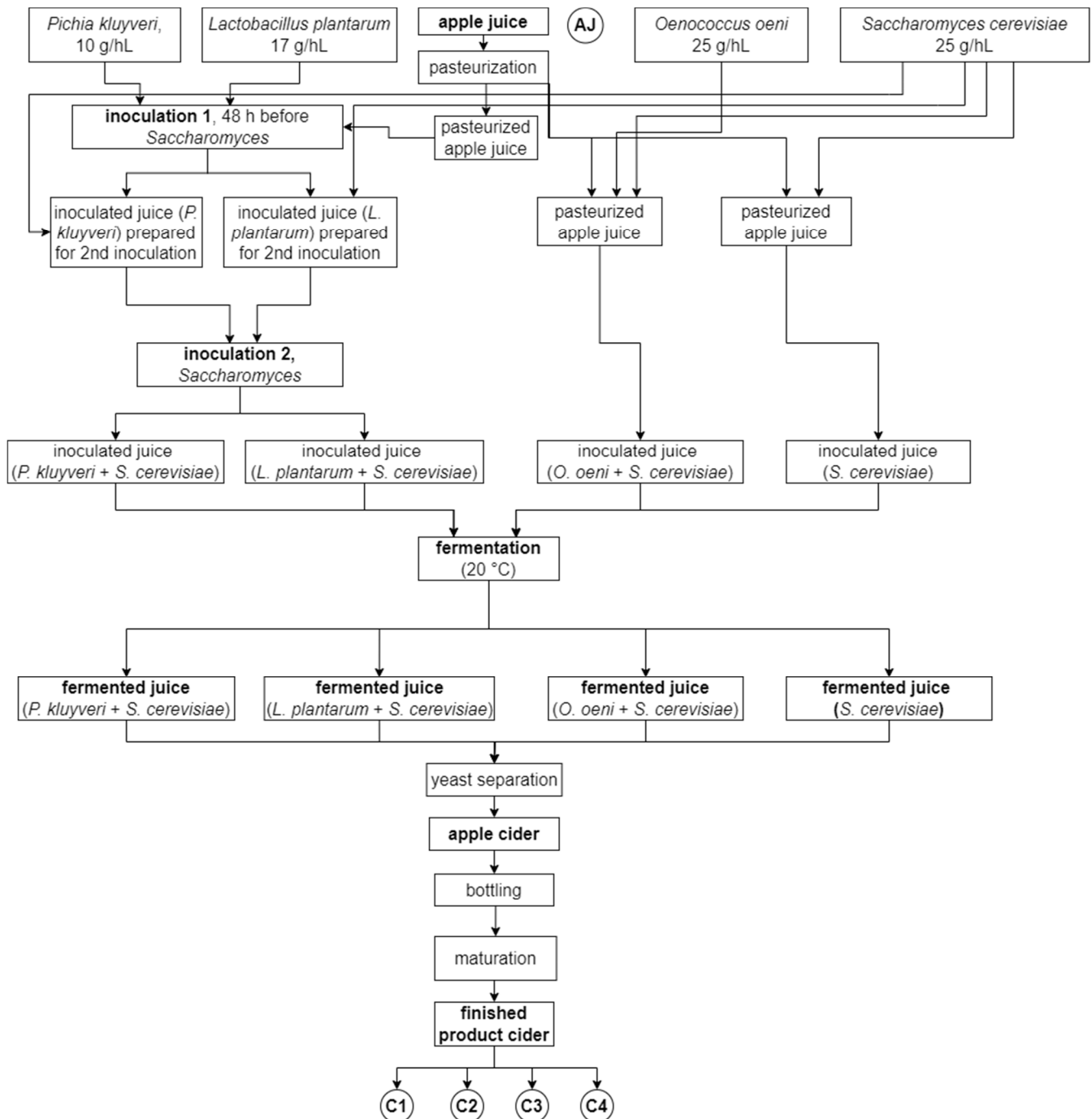

Figure 1. The experimental design and process flow of the apple cider production.

Equal amounts of AJ (11.1 °Brix, pH 3.46) were added to 2 disinfected stainless-steel fermenters, and then *P. kluyveri* (C1) and *L. plantarum* (C2), respectively, were inoculated in each of them. Based on the producers' instructions, *Saccharomyces* yeasts were inoculated in each variant after 48 h to continue the alcoholic fermentation. The same amount of juice was synchronously inoculated with *O. oeni* and *S. cerevisiae* (C3). *Saccharomyces* yeasts were

inoculated in the AJ as a control sample (C4). Fermentation took place in stainless-steel fermenters with a capacity of 25 L at a temperature of 20 °C. Monitoring of the Brix, pH and acidity occurred over the course of the 14-day fermentation process. Fermentation was considered completed when there was no difference in the °Brix values after 24 h and the number of viable yeasts in the fermented juice was below 1×10^5 CFU mL/L. The cider was clarified (10 days at 4 °C and decanted) towards the completion of fermentation and then bottled. Maturation took place at 10 °C for 120 days, and after this stage, the cider was subjected to chemical and sensory analyses.

The chemicals used were glucose, fructose and maltose (purity 99%); sulfuric acid 0.5 M (Chempur, Piekary Śląskie, Poland); ultrapure water purified with the Direct-Q UV system from Millipore (Burlington, MA, USA); malic, citric and succinic acids (99% purity, Merck, Germany); lactic and acetic acids (purity > 99%); pyruvic acid (98% purity) (Sigma-Aldrich, Merck, Germany); monosodium phosphate (NaH_2PO_4); dichlormethane (Merck, Germany); acetonitrile, of HPLC purity (Merck, Germany); gallic and chlorogenic acids (>98% HPLC grade); and rutin and catechin (>99% HPLC grade) (Sigma, Burlington, MA, USA). The EZ:Faast™ GC-FID amino acid analysis kit was provided from Phenomenex.

2.2. Glucides and Organic Acids Using HPLC

For the separation and quantification of glucides and lactic and acetic acids the procedure previously reported was followed [20]. Agilent ChemStation software version B.02.01.SR2 (Agilent Technologies, Santa Clara, CA, USA) was used to collect data and assess the results. The compounds in the examined samples were identified by comparing their retention times to those of the standard compounds [21]. The compounds were identified by comparing the retention times of the standards with those of the peaks from the apple juice and apple cider samples. All experiments were performed in triplicate.

2.3. Volatile Compounds Using GC/MS

The liquid–liquid extraction procedure used by Coelho et al. [22] for the sample was adapted with modifications, and ultrasonic extraction at 0 °C for 25 min was used instead of a magnetic stirrer. All extractions were carried out in triplicate.

The gas chromatographic analysis of the volatile compounds was performed using a GC-MS Shimadzu QP 2010 PLUS Mass Spectrometer coupled with a Gas Chromatograph (Shimadzu equipped with an AOC-20i+s injector, and a ZB-Wax MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Phenomenex, Torrance, CA, USA), as described in our previous experiments [20]. The results are expressed as a percentage of the total peak area (100%).

2.4. Amino Acids Using Gas-Chromatography

The samples were analyzed with gas chromatography using the Phenomenex EZ:Faast™ kit following one of our previous experiments [20]. For the analytical investigation, an Agilent 6890N (Agilent Technologies, Santa Clara, CA, USA) with a flame ionization detector (FID) was used. Additionally, the Zebron ZBAAA column was a 10 m × 0.25 mm capillary GC column. The temperature program on the column was: 30 °C/min from 110 °C to 320 °C. The FID temperature was 320 °C, and 2.5 μL was injected at a temperature of 250 °C and a split level of 1:15. The carrier gas was He at a pressure of 8 psi. Each sample was analyzed in duplicate. Data manipulation and processing were performed using Empower 2.0 software.

2.5. Analysis of Phenolic Compound Using HPLC-DAD ESI+

The analysis of the phenolic compound profiles of the apple juice and cider was performed as previously described by Coldea et al. [23] using an Agilent 1200 HPLC system equipped with a quaternary pump, solvent degasser, autosampler and UV-Vis detector with a photodiode (DAD) coupled with an Agilent model 6110 single quadrupole mass detector

(MS) (Agilent Technologies, Santa Clara, CA, USA). The separation of the compounds was carried out on a Kinetex XB C18 column (Phenomenex, Torrance, CA, USA) [24]. Data acquisition, interpretation of results and sample preparation were performed as described in Section 2.3. The phenolic compounds were identified by comparing their retention times, UV-Vis absorption and mass spectra with those of the standard compounds and the available literature data.

2.6. Sensory Analysis

The sensory analysis was performed at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The participants ($n = 7$, 4 men, 3 women) included a sommelier and two people working in quality control of alcoholic beverages, aged between 26 and 47 years. The selection criteria included their experience in beverage sensory analysis (minimum 2 years). The descriptive test of the flavor profile was performed using a 9 cm linear scale, where the raters recorded the sensation intensity, from "Imperceptible" (left edge) to "Very intense" (right end). Ratings were conducted to assess the intensity of the 13 attributes of visual appearance (clarity, color), smell (fruity, floral and yeasty), trigeminal sensations (astringency), taste (sour/acid, sweet, bitter) and aroma (fruity, floral and yeasty). Each apple cider sample (approximately 50 mL) was coded with three random digits and presented to the panel in wine glasses according to ISO 3591/1977 [25] at 8–10 °C.

2.7. Data Analysis

The data are reported as the average mean ± standard deviation (SD) for triplicate determinations. The statistical evaluation was performed with analysis of variance (ANOVA) using SPSS 19.0 software (IBM, New York, NY, USA) and Tukey's honest significant difference (HSD) test with a confidence interval of 95% or 99%; p-values less than 0.05 were considered statistically significant. Principal component analysis (PCA) (XLSTAT, 2021) was used to observe the correlation among samples with the identified compounds (polyphenols, amino acids, volatiles and sensory attributes) [26].

3. Results and Discussions

3.1. Determination of Sugars and Organic Acids

It is known that sugars (glucose and fructose) are converted during the alcoholic fermentation process into cellular energy, ethyl alcohol, carbon dioxide and other chemicals [27].

Glucose and fructose were found to be the two most abundant sugars in apple juice and cider samples, respectively, with the highest concentrations found in AJ, 42.54 and 61.11 g/L, respectively (Table 1). However, during fermentation, the levels of both sugars decreased considerably, falling below 5.5 g/L. As shown in previous studies [28], glucose was completely consumed by yeasts at the end of fermentation. Moreover, fructose concentrations decreased after fermentation to concentrations below 5.5 g/L in all variants. The fructose content of co-inoculated yeast–LABs ciders was twice as low as that of yeast-fermented ciders, because LABs use fructose to produce mannitol, lactic acid and acetic acid [29]. A high fructose consumption capacity of yeasts is essential for winemakers to tackle the difficulties caused by slow or stuck fermentations that result in unwanted sweetness in wines [30].

The polyol erythritol, with the highest content of 0.22 g/L in cider, was not present in apple juice, which is known to be a by-product of fermentation produced by *Saccharomyces* yeast strains [31] and LABs (*O. oeni* and *Lactobacillus* spp.) during malolactic fermentation [32]. However, it was formed during fermentation and single-strain fermentation with *Saccharomyces* yeast, possibly as a result of the heterofermentative routes that contribute to the metabolizing of glucose and fructose [31].

Both *Saccharomyces* and non-*Saccharomyces* yeasts, as well as mixed *Saccharomyces*-LAB fermentations, have the ability to produce sorbitol after the fermentation process [33]. Sorbitol influences the sweetness, smoothness and flavor complexity of the cider [34]. In

accordance with a previous study [35], it was observed that sorbitol recorded an extremely significant increase after the fermentation process, the highest increase of 1.67 g/L in single-strain yeast-fermented cider.

Table 1. Chemical parameters, sugars and organic acids in the apple juice and cider samples (g/L).

Compounds/Chemical Parameters	AJ	C1	C2	C3	C4	*p*-Value	Sig.
Ethanol (% *v/v*)	nd	6.10 ± 0.03 [b]	6.20 ± 0.02 [a]	6.20 ± 0.05 [a]	6.10 ± 0.01 [b]	$p < 0.01$	**
Total acidity (g L^{-1} malic acid)	6.61 ± 0.02 [a]	5.42 ± 0.03 [b]	3.14 ± 0.03 [d]	2.94 ± 0.03 [e]	4.28 ± 0.02 [c]	$p < 0.001$	***
pH	3.46 ± 0.04 [c]	3.55 ± 0.03 [c]	3.79 ± 0.03 [ab]	3.85 ± 0.04 [a]	3.71 ± 0.03 [b]	$p < 0.001$	***
Sugars							
Glucose	42.54 ± 0.56	nd	nd	nd	nd		
Fructose	61.11 ± 0.63 [a]	5.46 ± 0.27 [b]	1.85 ± 0.07 [d]	2.01 ± 0.09 [d]	3.79 ± 0,11 [c]	$p < 0.001$	***
Sorbitol	0.98 ± 0.05 [d]	1.01 ± 0.05 [d]	1.31 ± 0.06 [c]	1.44 ± 0.05 [b]	1.67 ± 0.06 [a]	$p < 0.001$	***
Erythritol	nd	0.10 ± 0.02 [c]	0.15 ± 0.03 [b]	0.17 ± 0.02 [ab]	0.22 ± 0.02 [a]	$p < 0.01$	**
Organic acids							
Malic	2.65 ± 0.11 [a]	0.14 ± 0.03 [b]	0.16 ± 0.02 [b]	0.18 ± 0.02 [b]	0.18 ± 0.02 [b]	$p < 0.05$	*
Lactic	nd	1.41 ± 0.08 [d]	2.83 ± 0.09 [b]	3.32 ± 0.10 [a]	2.23 ± 0.05 [c]	$p < 0.001$	***
Pyruvic	0.38 ± 0.04 [a]	0.33 ± 0.04 [ab]	0.30 ± 0.03 [ab]	0.24 ± 0.04 [b]	0.28 ± 0.03 [b]	$p < 0.05$	*
Citric	0.24 ± 0.02 [a]	0.11 ± 0.01 [c]	0.14 ± 0.02 [bc]	0.24 ± 0.03 [a]	0.19 ± 0.02 [b]	$p < 0.05$	*
Succinic	0.40 ± 0.03 [cd]	0.57 ± 0.02 [b]	0.35 ± 0.02 [d]	0.43 ± 0.02 [c]	0.65 ± 0.04 [a]	$p < 0.01$	**
Acetic	nd	0.07 ± 0.01 [b]	0.13 ± 0.03 [a]	0.15 ± 0.03 [a]	0.07 ± 0.01 [b]	$p < 0.05$	*

Values are expressed as the mean of three replicates. Values with different lowercase letters in the same row indicate statistically significant differences between samples (Tukey's test). * Significant, $p \leq 0.05$; ** very significant, $p \leq 0.01$; *** extremely significant, $p \leq 0.001$; ns = not significant; nd = not detected. AJ = apple juice; C1 = cider fermented with *P. kluyveri* + *S. cerevisiae*; C2 = cider fermented with *L. plantarum* + *S. cerevisiae*; C3 = cider fermented with *O. oeni* + *S. cerevisiae*; C4 = cider fermented with *S. cerevisiae*.

Figure 2 shows the PCA of the results obtained from the determination of carbohydrates in the analyzed cider samples. The scores obtained for the main component, F1, explain 85.46% of the variation of the samples, while for F2 only 14.46%. Basically, glucose and fructose together with sorbitol and erythritol achieve a clear differentiation of the ciders and apple juice, as samples C1 and C2 recorded lower concentrations of sorbitol and erythritol, respectively.

The major organic acid in the apple juice, malic acid, has a strong, bitter flavor and is commonly perceived as astringent and sour [36,37]. During alcoholic fermentation, yeasts can break down, with the production of lactic and succinic acids, amyl alcohol, isobutanol [38], and even malic acid [38]. The malic acid content in the apple juice was 2.65 g/L, while after fermentation it drastically reduced by more than 90% ranging between 0.14 and 0.18 g/L (Table 1). Malolactic fermentation, which is produced by lactic acid bacteria, converts dicarboxylic acid (i.e., malic acid) into monocarboxylic acid (i.e., lactic acid) and carbon dioxide, favoring a reduction in the acidity and astringency [21]. The reduction of pyruvic acid by LABs is another mechanism by which lactic acid is produced [39]. Lactic acid was not present prior to, but it was produced during fermentation along with a breakdown of the malic and pyruvic acids. Malolactic fermentation induced by *L. plantarum* and *O. oeni* facilitated an increase of lactic acid, in these variants, to 2.83 g/L and 3.32 g/L, respectively.

Succinic acid, another metabolite of alcoholic fermentation produced by yeasts, may also be found during malolactic fermentation as a result of the transformation of malic acid [38]. Additionally, succinic acid can be produced when amino acids break down [39]. Succinic acid was, in general, more abundant in the final cider compared to apple juice (0.40 g/L), except for *L. plantarum* + *S. cerevisiae*-fermented cider (C2). Succinic acid is essential in cider fermentation as it combines with other compounds and forms esters [38]. In the yeast-fermented ciders the amount of succinic acid was higher than in the cofermented

yeast–LAB ciders. This might be due to the decarboxylation of malic acid to lactic acid in a higher percentage in the samples with lactic acid bacteria, and the formation of a higher amount of succinic acid in the samples without LABs. Pyruvic acid, for instance, is an essential precursor for a variety of metabolites and is present in apple juice, but because it is produced by yeasts during fermentation, its concentration might vary [38]. Consistent with previous findings [40], pyruvic acid was present in lower concentrations in the apple cider (0.24–0.33 g/L) compared to the apple juice (0.38 g/L), as the increase in lactic acid is associated with a decline in pyruvic acid [41].

In the case of citric acid, it can be produced by *S. cerevisiae* during fermentation, but LABs further metabolize citric acid with the formation of lactic acid, diacetyl, acetoin and acetic acid [41]. These substances significantly affect the aroma of fermented beverages [42]. Further analysis of the cider samples showed that citric acid was present, with levels ranging from 0.11 g/L in C1 to 0.24 g/L in C3, similar to that of apple juice and much lower compared to previous findings [41].

After fermentation, the acetic acid concentration increased but remained below the perceptibility threshold which might give a pungent smell and a vinegar aroma to the cider, as stated in a previous study [43]. The key factors responsible for acetic acid's formation are the sugar composition and the source of nitrogen [44]. Because it is also the source of fruity acetates and the precursor of ethyl acetate, this acid plays a significant role in fermentation [8]. Acetic acid reached 0.13 and 0.15 g/L, respectively, in the LAB-cofermented apple ciders, similar to previously reported results for yeast-inoculated cider [41], and a much lower content in the yeast-fermented ciders (0.07 g/L).
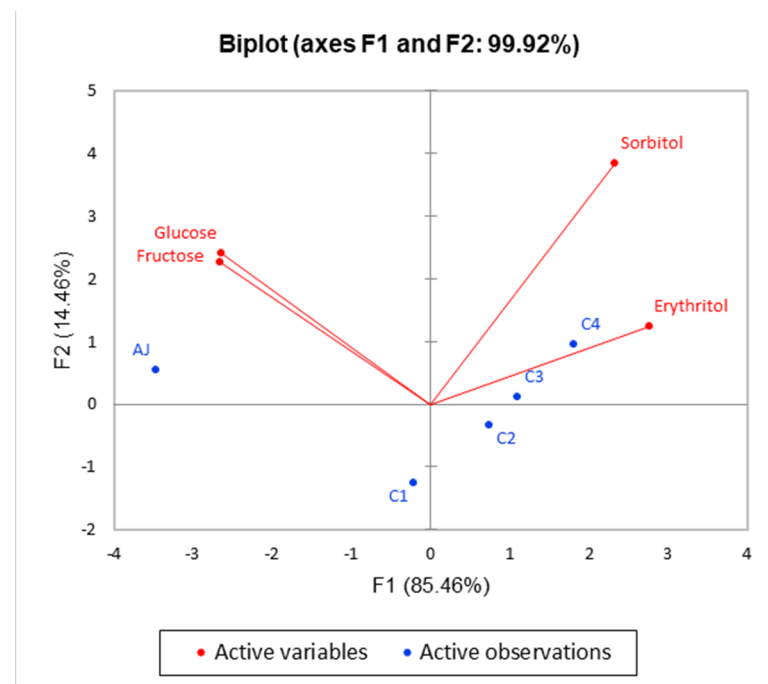

Figure 2. Scores plot of the principal component analysis (PCA) applied to the sugar analysis of the ciders and apple juice (F1 = first principal component; F2 = second principal component).

The PCA analysis of the organic acids (Figure 3) revealed that the main component, F1, had a value of 65.13%, while for F2 it was 21.70%, which explains the separation of AJ from the rest of the samples. Considering the concentration of the organic acids, the apple juice (AJ) was distinguished by a high content of malic acid. The co-fermented yeast–LABs ciders were differentiated by lactic and acetic acids, thus differentiating from yeast-inoculated ciders, respectively, which were grouped in the upper part, with a positive value for F2.
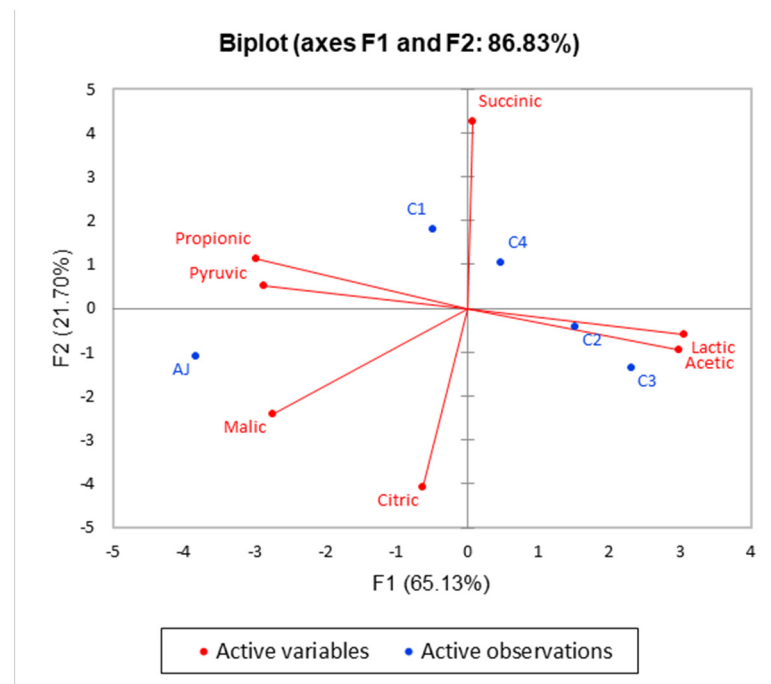
Figure 3. Scores plot of the principal component analysis (PCA) applied to the organic acids analysis of the ciders and apple juice (F1 = first principal component; F2 = second principal component).

3.2. Volatile Compounds Analysis

In our samples, a total of 32 volatile compounds were identified and quantified (Table 2). The volatile profiles of both the apple juice and cider could be influenced by a wide range of factors, such as the apple variety, fermentation method and yeast strains [45]. Based on the yeast strain and lactic acid bacteria utilized for the fermentation, it was observed that the volatile compound amounts varied significantly. Our study revealed a variety of volatile compounds, including fatty acids (e.g., hexanoic acid and octanoic acid), esters (e.g., ethyl acetate, ethyl 2-hydroxypropanoate, 3-methylbutyl acetate and ethyl octanoate), alcohols (e.g., butan-1-ol, 2-phenylethanol and 2-phenylethanol) and alkenes (2-methylbut-1-ene).

Esters are produced by reacting the corresponding higher alcohols with an acid to form an ester [46]. Even when in low amounts, they contribute to the fragrance of cider by giving the product typicity, such as a green apple flavor (hexyl acetate, generated from hexanol) [47], yellow apple aroma (ethyl-2-methylbutanoate) or fruity, strawberry, banana and caramel aromas (3-methylbutyl acetate). These abovementioned esters have a significant impact on the sensory profile of the cider [48]. 3-Methylbutyl acetate and butyl acetate confer a pear and berry, sweet and fruity flavor, respectively, to apple cider, but with relatively low aroma intensities [49]. Butyl acetate was found only in one single-strain yeast-fermented cider. As identified before in the single-strain yeast-fermented cider, ethyl octanoate imparts a fruity mainly apricot flavor [49,50]. We found it only in the yeast–*O. oeni*-fermented cider and in a low proportion.

Three fatty acids were found, namely, butanoic acid, octanoic acid and hexanoic acid, and they were mainly present in the yeast–LABs fermented ciders. All are recognized for their cheesy, fatty and sweet flavors offered to apple cider [49,50].

Alcoholic compounds also have a substantial impact on beverage sensory characteristics. Butane-2.3-diol, octane-1.3-diol, butan-1-ol and 2-phenylethanol were the most frequent alcoholic compounds found in the samples. The concentration of 3-methylbutan-1-ol among these compounds in the LAB co-fermented cider was the greatest (38.08 in C2 and 41.36 in C3). However, in the sample C1, the concentration was considerably lower. The butan-1-ol, hexan-1-ol and octane-1.3-diol concentrations in the cider samples were

lower and more similar than in the apple juice. As previously shown [50], one of the main compounds found in the apple cider in significant concentrations was 2-phenylethanol. It displayed a constant quantity in the samples fermented with LAB, similar to 1-butanol (Table 2).

Table 2. Volatile compounds found in apple juice and apple cider samples expressed as a percentage of the total peak area.

Volatile Compounds	AJ	C1	C2	C3	C4	*p*-Value	Sig.
2-Methylbut-1-ene	40.97 ± 2.81 [a]	27.79 ± 0.23 [b]	13.75 ± 0.21 [c]	15.67 ± 0.22 [c]	24.71 ± 2.53 [b]	$p < 0.01$	**
heptane	15.92 ± 1.56 [a]	15.91 ± 2.36 [a]	11.10 ± 0.33 [b]	12.82 ± 0.32 [ab]	16.34 ± 1.47 [a]	$p < 0.01$	**
2-Methylpentan-3-ol	3.56 ± 0.07 [a]	2.01 ± 0.33 [b]	1.53 ± 0.04 [c]	2.07 ± 0.04 [b]	2.30 ± 0.10 [b]	$p < 0.01$	**
butyl acetate	3.36 ± 0.29 [a]	0.81 ± 0.08 [b]	0.29 ± 0.01 [c]	0.47 ± 0.03 [c]	nd	$p < 0.001$	***
2-Methylpropan-1-ol	nd	2.30 ± 0.35 [b]	2.60 ± 0.04 [ab]	2.74 ± 0.06 [a]	2.62 ± 0.06 [ab]	$p < 0.05$	*
3-Methylbutyl acetate	nd	2.72 ± 0.27 [a]	0.82 ± 0.03 [b]	nd	nd	$p < 0.001$	***
butan-1-ol	6.03 ± 0.69 [a]	1.33 ± 0.22 [b]	1.66 ± 0.02 [b]	1.67 ± 0.03 [b]	1.58 ± 0.08 [b]	$p < 0.01$	**
3-Methylbutan-1-ol	nd	31.44 ± 2.32 [c]	39.08 ± 0.35 [ab]	41.36 ± 0.20 [a]	37.10 ± 0.51 [b]	$p < 0.001$	***
2-Methylbutan-1-ol	0.94 ± 0.23	nd	nd	nd	nd		
hexyl acetate	1.48 ± 0.27 [a]	0.22 ± 0.04 [b]	0.21 ± 0.02 [b]	nd	nd	$p < 0.01$	**
3-Hydroxybutan-2-one	nd	nd	0.31 ± 0.02	nd	nd		
ethyl 2-hydroxypropanoate	nd	nd	0.52 ± 0.02 [b]	3.54 ± 0.05 [a]	nd	$p < 0.001$	***
Hexan-1-ol	3.46 ± 0.44 [a]	0.41 ± 0.03 [c]	0.61 ± 0.02 [bc]	0.98 ± 0.03 [b]	0.86 ± 0.04 [bc]	$p < 0.001$	***
Butane-2.3-diol	nd	0.23 ± 0.04 [c]	1.49 ± 0.03 [a]	1.05 ± 0.02 [b]	nd	$p < 0.001$	***
2-Methylpropanoic acid	nd	0.25 ± 0.05 [a]	0.16 ± 0.02 [b]	0.21 ± 0.04 [ab]	nd	$p < 0.01$	**
Butanoic acid	nd	0.23 ± 0.04 [a]	0.25 ± 0.03 [a]	nd	nd	$p > 0.05$	ns
Hexanoic acid	nd	0.38 ± 0.06 [b]	0.67 ± 0.04 [a]	0.70 ± 0.04 [a]	nd	$p < 0.01$	**
2-Methylbutanoic acid	nd	nd	0.21 ± 0.03 [a]	0.22 ± 0.01 [a]	nd	$p > 0.05$	ns
3-Methylsulfanylpropan-1-ol	nd	0.39 ± 0.03 [c]	0.63 ± 0.04 [a]	0.55 ± 0.02 [b]	nd	$p < 0.001$	***
Ethyl acetate	nd	nd	0.25 ± 0.01 [a]	0.22 ± 0.01 [a]	nd	$p > 0.05$	ns
Methyl 4-hydroxybutanoate	nd	nd	0.41 ± 0.02 [b]	0.37 ± 0.02 [b]	0.85 ± 0.04 [a]	$p < 0.01$	**
2-Phenylethyl acetate	nd	2.00 ± 0.03 [a]	0.88 ± 0.02 [b]	nd	nd	$p < 0.001$	***
2-Phenylethanol	nd	5.12 ± 0.22 [c]	8.09 ± 0.15 [b]	9.54 ± 0.07 [a]	8.23 ± 1.12 [ab]	$p < 0.001$	***
Octanoic acid	nd	nd	0.89 ± 0.04 [b]	1.08 ± 0.05 [ab]	1.31 ± 0.25 [a]	$p < 0.05$	*
Octane-1.3-diol	9.62 ± 0.66 [a]	1.77 ± 0.28 [c]	2.71 ± 0.09 [b]	3.35 ± 0.07 [b]	2.63 ± 0.09 [bc]	$p < 0.01$	**
Pentan-2-ol	1.33 ± 0.18	nd	nd	nd	nd		
Heneicosane	1.75 ± 0.11	nd	nd	nd	nd		
Ethyl 3-methylbutanoate	nd	0.21 ± 0.01	nd	nd	nd		
2-Butoxyethyl acetate	nd	0.15 ± 0.01	nd	nd	nd		
Ethyl octanoate	nd	nd	nd	0.18 ± 0.02	nd		
3-Methylsulfanylpropan-1-ol	nd	nd	nd	nd	0.53 ± 0.02		
Butyl acetate	nd	nd	nd	nd	0.30 ± 0.04		

Values are expressed as the mean of three replicates. Values with different lowercase letters in the same row indicate statistically significant differences between samples (Tukey's test). * Significant, $p \leq 0.05$; ** very significant, $p \leq 0.01$; *** extremely significant, $p \leq 0.001$; ns = not significant; nd = not detected. AJ = apple juice; C1 = cider fermented with *P. kluyveri* + *S. cerevisiae*; C2 = cider fermented with *L. plantarum* + *S. cerevisiae*; C3 = cider fermented with *O. oeni* + *S. cerevisiae*; C4 = cider fermented with *S. cerevisiae*.

Amino acids are a nitrogen source for yeasts. They have a great impact on the amount of higher alcohols formed during fermentation via the Ehrlich pathway [51]. Based on their origins as amino acids, these compounds can be divided into three groups: branched-chain, sulfur-containing, and aromatic alcohols. Higher alcohols make a favorable contribution to alcoholic beverages' overall complexity at lower percentages [52]. Hexan-1-ol and 2-methylpropan-1-ol were among the most significant higher alcohols in cider, which provide a pleasant and sweet flavor, while 3-methylbutanol adds fruity aromas [49]. It was observed that the amounts of esters, including butyl acetate, ethyl acetate, hexyl acetate and other ethyl esters, varied according to the species of yeast and LAB utilized in the fermentation process. The fruity and floral aromas of the cider were mostly attributed to these compounds, which yeasts produce during fermentation. 2-Butoxyethyl acetate, also known as methylbutanoate, was found only in the *Pichia*-fermented apple cider. *P. kluyveri*

was previously reported for its advantage of the increase in the variety of esters and thiols provided through fermentation [6].

The PCA (Figure 4) showed that the AJ sample obviously differed from all other studied samples and had the lowest value for F1. The yeast-fermented ciders, which recorded diametrically opposed positions for F2, were distinguished from the other samples. This indicates that *Pichia*, utilized in association with *Saccharomyces*, clearly influenced the volatile profiles of the ciders. Although the yeast–LAB cofermented ciders are in distinct quadrants relative to F2, they are situated much closer together and had similarly more volatile compounds as a consequence of the similar contribution to their volatile profiles of 2-methylbut-1-ene, hexanoic acid, 2-methylbutanoic acid, 3-methylsulfanylpropan-1-ol, ethyl acetate and methyl 4-hydroxybutanoate.
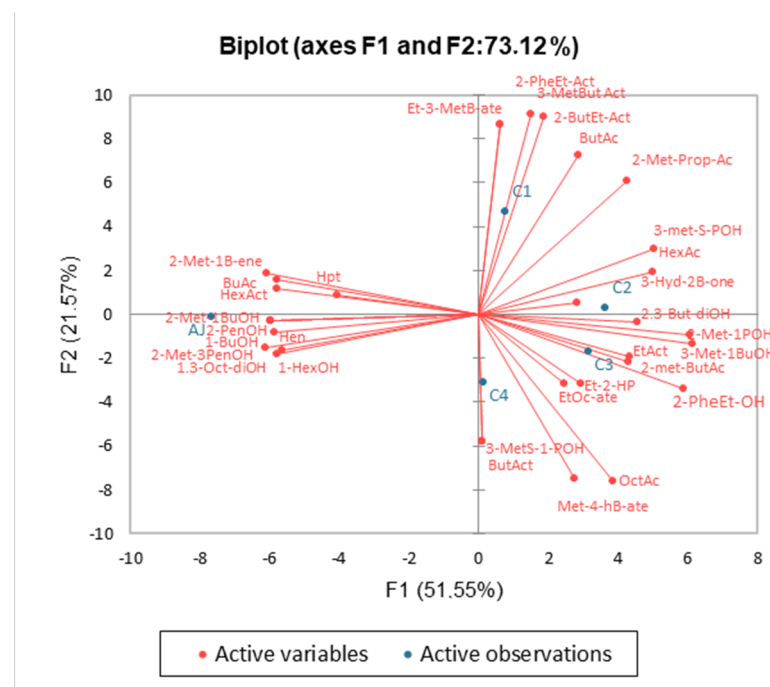

Figure 4. Scores plot of the principal component analysis (PCA) applied to the volatile compounds analysis of the ciders and apple juice (F1 = first principal component; F2 = second principal component). 2-Met-1B-ene = 2-methylbut-1-ene; Hpt = Heptane; 2-Met-3PenOH = 2-methylpentan-3-ol; BuAc = butyl acetate; 2-Met-1POH = 2-methylpropan-1-ol; 3-MetBut Act = 3-methylbutyl acetate; 1-BuOH = butan-1-ol; 3-Met-1BuOH = 3-methylbutan-1-ol; 2-Met-1BuOH = 2-methylbutan-1-ol; Hex-Act = hexyl acetate; 3-Hyd-2B-one = 3-hydroxybutan-2-one; Et-2-HP = ethyl 2-hydroxypropanoate; 1-HexOH = hexan-1-ol; 2.3-But-diOH = butane-2.3-diol; 2-Met-Prop-Ac = 2-methylpropanoic acid; ButAc = Butanoic acid; HexAc = Hexanoic acid; 2-met-ButAc = 2-methylbutanoic acid; 3-met-S-POH = 3-methylsulfanylpropan-1-ol; EtAct = Ethyl Acetate; Met-4-hB-ate = Methyl 4-hydroxybutanoate; 2-PheEt-Act = 2-phenylethyl acetate; 2-PheEt-OH = 2-phenylethanol; OctAc = Octanoic Acid; 1.3-Oct-diOH = octane-1.3-diol; 2-PenOH = pentan-2-ol; Hen = Heneicosane; Et-3-MetB-ate = ethyl 3-methylbutanoate; 2-ButEt-Act = 2-butoxyethyl acetate; EtOc-ate = ethyl octanoate; 3-MetS-1-POH = 3-methylsulfanylpropan-1-ol; ButAct = butyl acetate.

3.3. Amino Acids Profiles of the Ciders and Apple Juice

Amino acids (AAs) are the nutrient source needed for the growth and development of yeasts during fermentation and constitute the yeast assimilable nitrogen that is naturally present in apple juice [53]. Climate conditions and apple varieties have a direct impact on their amount in apples [54]. The easiest amino acids for yeast to assimilate are asparagine, aspartate, glutamic acid, alanine and serine, which constitute over 80% of the total amino acids in apple juice [55]. The ciders' amino acids (AAs) profile included 14 amino acids

(Table 3). The cofermented ciders had a substantially lower concentration (under 18 mg/L), but the single-strain fermentation cider had the highest total amino acid concentration of 50.84 mg/L. As stated in previously published studies [43,56], asparagine, aspartic acid, glutamic acid, α-aminoadipic acid and alanine were the primary amino acids found in apple juice, with asparagine having the highest concentration (287.76 mg/L) and providing a high consumption during the fermentation. These findings support previous work regarding the amino acid composition in apple juice, which reported that the amino acid composition affects the formation of volatile compounds that contribute to the flavor of fermented beverages [57]. The results show that the content of AAs decreased by between 88% and 96% after the fermentation process. As shown before [41], the utilization of amino acids as the main supply of nitrogen, which promotes yeast's normal growth and speeds up the process of alcoholic fermentation, can be attributed to this decrease. The information in Table 3 clearly shows that cofermentation (C1, C2 and C3) consumed more amino acids than single-strain fermentation (C4).

Table 3. Amino acids (mg/L) in the apple juice and ciders.

Amino Acids	AJ	C1	C2	C3	C4	*p*-Value	Sig.
Alanine	16.6 ± 0.52 [a]	6.2 ± 0.16 [c]	4.55 ± 0.12 [d]	5.9 ± 0.13 [c]	13.07 ± 0.17 [b]	$p < 0.001$	***
Isoleucine	3.21 ± 0.08	nd	nd	nd	nd		
Serine	8.35 ± 0.17	nd	nd	nd	nd		
Proline	2.13 ± 0.09	nd	nd	nd	nd		
Asparagine	287.76 ± 4.82 [a]	3.61 ± 0.16 [b]	nd	nd	2.78 ± 0.08 [b]	$p < 0.01$	**
Aspartate	58.96 ± 1.16	nd	nd	nd	nd		
Glutamic acid	44.68 ± 1.44	nd	nd	nd	nd		
Phenylalanine	nd	1.55 ± 0.11 [b]	2.01 ± 0.17 [a]	1.99 ± 0.10 [a]	1.93 ± 0.06 [a]	$p < 0.05$	*
α-Aminoadipic acid	24.42 ± 0.86	nd	nd	nd	nd		
Glutamine	nd	nd	nd	nd	5.00 ± 0.20		
Lysine	3.85 ± 0.09 [a]	3.89 ± 0.17 [a]	3.16 ± 0.17 [b]	3.25 ± 0.20 [b]	2.24 ± 0.15 [c]	$p < 0.01$	**
Histidine	nd	nd	3.88 ± 0.09 [a]	3.92 ± 0.09 [a]	3.95 ± 0.14 [a]	$p > 0.05$	ns
Tyrosine	nd	nd	nd	nd	1.26 ± 0.07		
Tryptophan	nd	2.28 ± 0.08 [b]	2.66 ± 0.14 [a]	2.81 ± 0.17 [a]	2.74 ± 0.14 [a]	$p < 0.05$	*

Values are expressed as the mean of three replicates. Values with different lowercase letters in the same row indicate statistically significant differences between samples (Tukey's test). * Significant, $p \leq 0.05$; ** very significant, $p \leq 0.01$; *** extremely significant, $p \leq 0.001$; ns = not significant; nd = not detected. AJ = apple juice; C1 = cider fermented with *P. kluyveri* + *S. cerevisiae*; C2 = cider fermented with *L. plantarum* + *S. cerevisiae*; C3 = cider fermented with *O. oeni* + *S. cerevisiae*; C4 = cider fermented with *S. cerevisiae*.

Aspartic acid, glutamic acid, α-aminoadipic acid, serine, isoleucine and proline were entirely consumed, while 98% of asparagine was consumed in C1 and C4, and it was completely consumed in C2 and C3. On the other hand, some amino acids (tryptophan, phenylalanine, histidine and tyrosine) considerably increased in the final ciders, possibly as a result of a yeast's strain-specific metabolism or the transformation of other amino acids [43].

According to the results obtained by PCA analysis (F1—74.5% and F2—21.15%, respectively) (Figure 5), apple juice (AJ) and C4 were the most distinguished from the other analyzed samples. It is important to mention that the C2 and C3 samples overlapped, which makes them almost identical in terms of amino acid profile. As mentioned before, the volatile profile of the LAB–yeast fermented ciders was similar (Figure 4), and the same trend was observed in the amino acid consumption (Table 3, Figure 5) for the same cider variants.
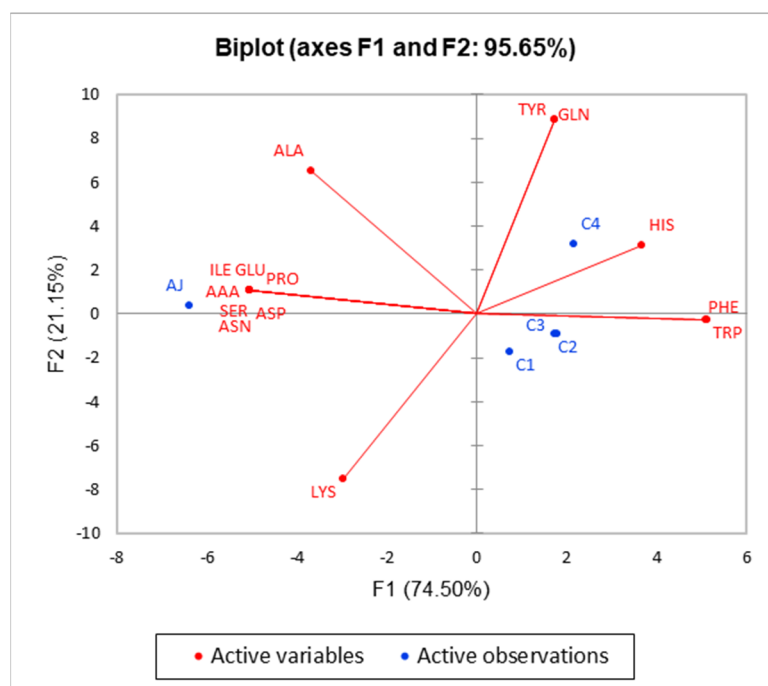
Figure 5. Scores plot of the principal component analysis (PCA) applied to the amino acids analysis of ciders and apple juice (F1 = first principal component; F2 = second principal component). ALA = Alanine; ILE = Isoleucine; SER = Serine; PRO = Proline; ASN = Asparagine; ASP = Aspartate; GLU = Glutamic acid; PHE = Phenylalanine; AAA = α-Aminoadipic acid; GLN = Glutamine; LYS = Lysine; HIS = Histidine; TYR = Tyrosine; TRP = Tryptophan.

3.4. Phenolic Compounds Analysis

Polyphenols are correlated to the cider aroma, some of them are precursors of volatile compounds, as well as color stability, because they play a role in oxidation reactions, flavoring and bitterness [58]. Flavonoids, dihydrochalcones, anthocyanidins and hydroxycinnamic acids are phenolics present in the apple fruit [59]. The quantity of phenols in the apple juice utilized in our research was 623.22 mg/L. As such, 20 phenolic compounds were identified and quantified in apple juice and cider. Following the fermentation phase, the fluctuation of phenolic compounds from the apple juice was observed (Table 4). The significant changes in phenolic compounds from apple juice may be due to the polyphenol enzymatic conversion during the fermentation process. Polyphenols are transformed by an enzymatic process into small molecules of phenolic compounds with higher biological activity [60]. Similar to the amounts of phenolic composition reported before in apple cider [35], the single yeast strain fermented cider (C4) had the highest quantity of phenolic compounds (522.99 mg/L), while *Saccharomyces + O. oeni* and *Saccharomyces + L. plantarum* had 419.66 mg/L, and 373.26 mg/L, respectively. However, it is important to highlight that the cofermented cider *Pichia + Saccharomyces* showed lower amounts of phenolic compounds, with values of 310.08 mg/L, which were half compared to those in the apple juice.

Independent of the apple variety but strongly influenced by the yeast strains used in the fermentation process, chlorogenic acid represents one of the major organic acids present in apple cider [61]. Ranging between 153.05 and 248.28 mg/L, it was predominate among the polyphenols in the analyzed cider samples followed by epicatechin, procyanidin dimer B2, catechin and procyanidin trimer C1 (Table 4). Chlorogenic acid, p-coumarylquinic acid and epicatechin were previously proved to be the main contributors to the antioxidant activity of apple cider [62]. Despite their antioxidant activity and the capability to prevent many diseases, phenolic compounds may modify the sensory profile of cider [35]. For example, catechin and epicatechin were previously reported as being correlated to the fruit aroma of apple cider [63], while procianidin B2, epicatechin and catechin were also found to

contribute to an astringent and bitter taste [64]. Some of the phenolic compounds exhibited an increase in the cider compared to apple juice, such as 2,3-dihydroxybenzoic acid that increased from 6.08 g/L in apple juice to 9.45 g/L in the single-strain yeast-fermented cider (C4) and over 8.3 g/L in both yeast–LAB fermented ciders. Other polyphenols, such as gentisic acid and catechin, exhibited slight increases in samples C3 and C4. Gallic acid and protocatechuic acid decreased below 0.1 mg/L in C1 and C3, respectively, in C1, C2 and C3. The only phenolic acid that was constant in the apple juice and all cider samples was procyanidin trimer C1, and its variation among samples was less than 5% (Table 4).

Table 4. Phenolic compounds (mg/L) in the apple juice and apple ciders.

Phenolic Compounds	AJ	C1	C2	C3	C4	*p*-Value	Sig.
p-Anisaldehida	21.01 ± 1.35 [a]	2.41 ± 0.04 [b]	0.13 ± 0.01 [cd]	1.68 ± 0.04 [bc]	0.04 ± 0.01 [d]	$p < 0.001$	***
Gallic acid-glucoside	6.55 ± 0.16 [a]	1.71 ± 0.06 [c]	1.41 ± 0.06 [d]	0.96 ± 0.05 [e]	4.51 ± 0.10 [b]	$p < 0.001$	***
2,3-Dihydroxybenzoic acid	6.08 ± 0.19 [c]	3.73 ± 0.12 [d]	8.75 ± 0.13 [b]	8.32 ± 0.18 [b]	9.45 ± 0.26 [a]	$p < 0.001$	***
Gallic acid	2.81 ± 0.08 [a]	0.04 ± 0.01 [d]	0.33 ± 0.03 [c]	0.07 ± 0.01 [d]	0.84 ± 0.05 [b]	$p < 0.001$	***
Gentisic acid	2.92 ± 0.10 [c]	2.42 ± 0.10 [d]	1.62 ± 0.09 [e]	3.47 ± 0.09 [b]	3.94 ± 0.10 [a]	$p < 0.001$	***
Protocatechuic acid	1.65 ± 0.07 [b]	0.07 ± 0.01 [d]	0.09 ± 0.02 [d]	0.67 ± 0.04 [c]	2.27 ± 0.05 [a]	$p < 0.001$	***
Neochlorogenic acid	9.81 ± 0.13 [a]	3.40 ± 0.04 [e]	4.86 ± 0.15 [d]	5.27 ± 0.13 [c]	6.81 ± 0.16 [b]	$p < 0.001$	***
Procyanidin dimer B1	26.86 ± 0.75 [a]	13.08 ± 0.43 [d]	16.37 ± 0.83 [c]	14.87 ± 0.88 [cd]	22.49 ± 1.05 [b]	$p < 0.001$	***
Catechin	24.86 ± 0.98 [b]	19.58 ± 0.56 [c]	24.35 ± 0.86 [b]	26.06 ± 1.55 [b]	30.97 ± 1.25 [a]	$p < 0.001$	***
Chlorogenic acid	312.69 ± 4.63 [a]	153.05 ± 3.53 [e]	174.07 ± 3.36 [d]	210.62 ± 3.10 [c]	248.28 ± 5.57 [b]	$p < 0.001$	***
Procyanidin dimer B2	50.60 ± 1.99 [a]	22.89 ± 0.73 [d]	29.51 ± 0.82 [c]	27.03 ± 2.30 [cd]	43.39 ± 1.67 [b]	$p < 0.001$	***
Epicatechin	43.55 ± 1.99 [a]	25.13 ± 1.33 [c]	37.80 ± 1.62 [b]	38.63 ± 0.91 [b]	46.38 ± 1.35 [a]	$p < 0.001$	***
p-Coumaroylquinic acid	30.72 ± 1.11 [a]	11.49 ± 0.65 [d]	16.63 ± 0.92 [c]	22.98 ± 1.54 [b]	29.63 ± 0.96 [a]	$p < 0.001$	***
Quercetin-rutinoside	5.97 ± 0.26 [a]	2.26 ± 0.09 [d]	3.56 ± 0.36 [c]	3.84 ± 0.12 [c]	5.09 ± 0.22 [b]	$p < 0.001$	***
Quercetin-glucoside	5.44 ± 0.12 [a]	2.33 ± 0.04 [d]	3.11 ± 0.08 [c]	3.15 ± 0.11 [c]	5.18 ± 0.09 [b]	$p < 0.001$	***
Quercetin-arabinoside	5.66 ± 0.16 [a]	1.81 ± 0.10 [d]	2.41 ± 0.10 [c]	2.64 ± 0.09 [c]	3.63 ± 0.09 [b]	$p < 0.001$	***
Phloretin-xylosyl-glucoside	27.49 ± 0.85 [a]	11.82 ± 0.09 [d]	12.69 ± 0.33 [cd]	13.37 ± 0.37 [c]	20.19 ± 0.63 [b]	$p < 0.001$	***
Quercetin-(malonyl-glucoside)	10.30 ± 0.23 [a]	5.38 ± 0.15 [d]	6.36 ± 0.43 [c]	7.26 ± 0.25 [b]	7.97 ± 0.39 [b]	$p < 0.001$	***
Phloridzin	3.81 ± 0.13 [b]	2.11 ± 0.07 [d]	4.28 ± 0.11 [b]	3.24 ± 0.17 [c]	6.60 ± 0.32 [a]	$p < 0.001$	***
Procyanidin trimer C1	24.44 ± 1.24 [a]	25.34 ± 1.29 [a]	24.93 ± 1.27 [a]	25.52 ± 1.14 [a]	25.32 ± 0.85 [a]	$p > 0.05$	ns

Values are expressed as the mean of three replicates. Values with different lowercase letters in the same row indicate statistically significant differences between samples (Tukey's test). *** extremely significant, $p \leq 0.001$; ns = not significant; nd = not detected. AJ = apple juice; C1 = cider fermented with *P. kluyveri* + *S. cerevisiae*; C2 = cider fermented with *L. plantarum* + *S. cerevisiae*; C3 = cider fermented with *O. oeni* + *S. cerevisiae*; C4 = cider fermented with *S. cerevisiae*.

The apple juice (AJ) differentiated itself the most from the other analyzed samples, along with the other classes of compounds, according to the results of the PCA analysis. Additionally, in terms of the phenolic composition, the ciders fermented with different yeast strains were very distinct from each other, while the yeast–LAB fermented ciders were similar and corresponded to the same quadrant (Figure 6).

3.5. Sensory Analysis

The flavor profile test is a descriptive test that is performed with expert assessors with the aim of fully describing the sample from a sensory perspective, including the perceived product defects. The results provide the capacity to compare and categorize cider samples based on their sensory characteristics examined. As a general remark, any sample can be described by a variety of characteristics. Because cider is a fermented product, its sour or acidic taste is a basic sensory quality. Additionally, the yeast flavor in samples may be more or less strong.

A PCA was used to identify relationships between the samples and sensory qualities and to observe trends in the data. A clear differentiation can be observed among the samples in terms of organoleptic characteristics, which can be attributed to the chemical composition of the cider (Figure 7). Firstly, the C1 sample produced by cofermentation of *Pichia* + *Saccharomyces* is distinguished by its sweet flavor and apple aroma, as well as other

fruit aromas. It is confirmed, in accordance with other studies [5,65], that using *P. kluyveri* yeast has the advantage of producing aromatic volatile compounds, which impact the sensory profile of the beverage.
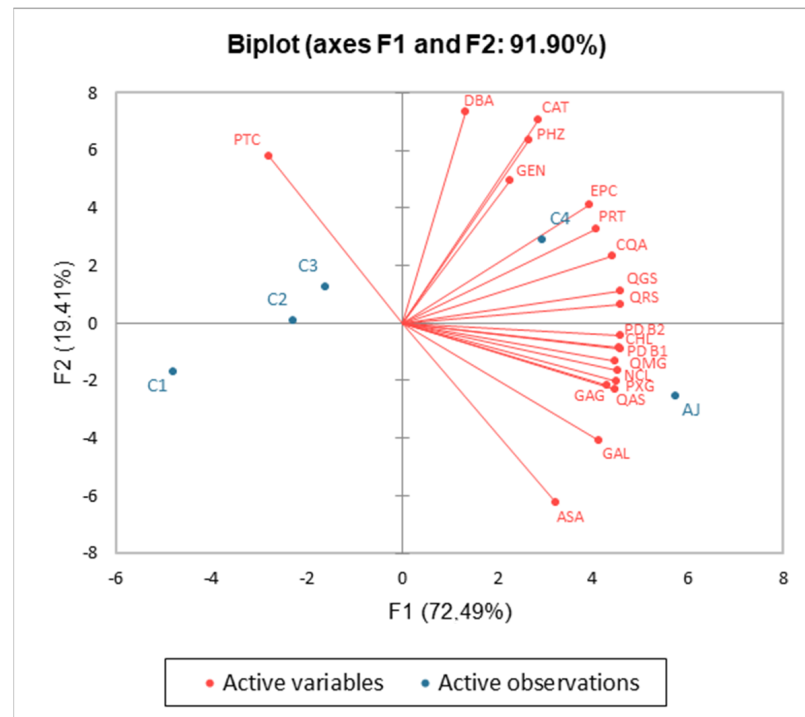

Figure 6. Scores plot of the principal component analysis (PCA) applied to the phenolic compounds analysis of the apple juice and cider samples. ASA = *p*-Anisaldehida; GAG = Gallic acid-glucoside, DBA = 2,3-Dihydroxybenzoic acid; GAL = Gallic acid; GEN = Gentisic acid; PRT = Protocatechuic acid; NCL = Neochlorogenic acid; PD B1 = Procyanidin dimer B1; CAT = Catechin; CHL = Chlorogenic acid; PD B2 = Procyanidin dimer B2; EPC = Epicatechin; CQA = p-Coumaroylquinic acid; QRS = Quercetin-rutinoside; QGS = Quercetin-glucoside; QAS = Quercetin-arabinoside; PXG = Phloretin-xylosyl-glucoside; QMG = Quercetin-(malonyl-glucoside); PHZ = Phloridzin; PTC = Procyanidin trimer C1.

Following the fermentation process, the cider obtained may also contain a considerable amount of malic acid, which can make the beverage sour and astringent. Malolactic fermentation leads to the formation of lactic acid, which is more delicate and can give the drink a more desirable flavor profile. In this regard, yeast–LAB fermented ciders are characterized by pleasant, mild aromas and odors, as well as pleasant aftertastes. The single-strain fermented cider (C4) was noted for its high polyphenol content that also conferred a more bitter taste, a fact also confirmed by the available literature [66].
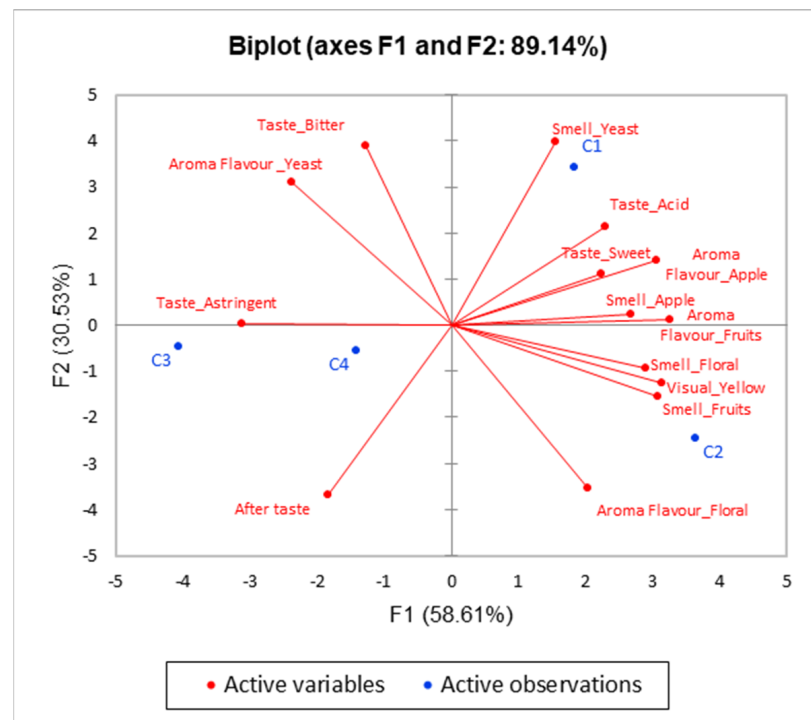
Figure 7. Scores plot of the principal component analysis (PCA) of the aroma profile performed on cider samples.

4. Conclusions

Our study outlined the effect of simultaneous fermentations of *Saccharomyces + Pichia kluyveri* and LAB + *Saccharomyces* on the chemical composition of apple cider obtained from two apple varieties: Topaz and Red Topaz. The amino acid content of the apple juice was within the scope of need. The level of the phenolic compounds was also high, which relays the technological demands of the ciders. Independent of the LAB strain used in the simultaneous yeast–LAB fermentation, the ciders had a similar amino acid consumption that also conferred similar phenolic and volatile profiles. The residual fructose imparts undesired sweetness in apple cider. The highest fructose consumption was also achieved by the yeast–LAB fermented ciders. When *L. plantarum* and *O. oeni* were used, this facilitated an increase in the lactic acid in the apple cider and the pleasant, mild aromas and odors and pleasant aftertaste. A stronger bitter taste was perceived in the single-strain fermented cider. The cofermented *Pichia + Saccharomyces* apple cider was distinguished by its sweet flavor and apple aroma, as well as other fruity aromas. Based on our findings, cider producers may select from various yeast–LAB strains to acquire specific phenolic, volatile and sensory profiles for their products in a more sustainable and time-efficient manner.

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

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