Article

New Ciders Made by an Exhaustion Method: An Option to Val-Orise Subproducts from the Making of Ice Ciders

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Abstract: Cryo-extraction (pressing of frozen apples), is one of the two freeze-enrichment systems allowed for the making of ice juices. Its ciders are often described as more complex and aromatic, however, the production yield is quite low. The Exhaustion method associated with the previous one proposes the valorisation of the discarded apple juice fractions for the making of new ciders. Three types of apple juices and three species of yeasts (*S. bayanus*, C6; *S. cerevisiae*, Levuline-CHP, and *T. delbrueckii*, Biodiva-TD291) have been used to evaluate the Exhaustion method. The ciders obtained were analysed for chemical and volatile composition as well as sensory characteristics. The yield (%) of the Exhaustion process ranged between 24 and 37%. The yeasts promoted the fermentation at different rates, providing ciders with alcoholic degrees between 9 and 12 (% v/v), and low volatile acidities. The yeast strain significantly influenced most of the parameters analysed, whereas the raw apple juice influenced the perception of the attributes fruity, apple and butter. Although the ciders produced by Exhaustion presented significantly lower concentrations of all the volatile compounds analysed than the corresponding ice ciders obtained by Cryo-extraction, the *S. bayanus* C6 and *T. delbrueckii* TD291 gave highly valued ciders from the sensory point of view.

Keywords: freeze-enrichment; Cryo-extraction; yeast strains; apple juice; alcoholic fermentation; volatile compounds; sensory analysis

1. Introduction

Cider is the alcoholic beverage obtained from the total or partial fermentation of the juices coming from freshly pressed apples or reconstituted apple concentrates. Nowadays, more than 60% of the world's cider production and consumption comes from Europe, clearly led by the United Kingdom; across the Atlantic Ocean, Argentina and the USA are the world's most important cider producers [1,2]. The global cider market is projected to value USD 6.56 billion by the end of 2024, registering a compound annual growth rate CAGR of 5.30% during the period between 2019–2024. This market expansion can be explained by the growing consumer demand for low alcohol and innovative fruity beverages, cider being perceived as healthier than other alcoholic drinks [3,4]. Moreover, in North America and Australia, there is an increasing preference towards small breweries making craft ciders–linked to locally harvested fresh-market apples–and specialty hard ciders made with cider apples, which may involve new economic opportunities for both apple growers and cider-makers [5–7]. This burgeoning and dynamic market trend coexists with commercialization schemes linked to geographical protection figures in countries having a long cider heritage, and with the making of exclusive products of very high economic value.

Ice cider is a unique appetizer and dessert apple wine made by the partial fermentation of high-density apple musts enriched by freezing. This beverage was first developed in Canada in the 90s, its success spreading rapidly worldwide ever since. Leading players in

the ice cider market are enterprises from Canada and the USA, followed by Sweden and Poland [8]. Nevertheless, this type of cider is also made in other countries.

Ice cider is characterised by its intense sweetness and acidity, smooth mouthfeel, and aromas described as fruity, ripe apple, candy, exotic fruits, and honey [9,10]. Legislative regulations state the minimum residual sugar concentration (100–130 g/L) and alcoholic degree (7–8% v/v) required for the labelling of a product as an ice cider [10,11] and, thus, apple juices with high-sugar contents, obtained by using cold temperatures, are necessary as the starting raw material. The regulations allow two freeze-enrichment processes to be used to obtain the ice apple juices: Cryo-concentration, the most frequently used, consists of pressing the apples and freezing the resulting juice; Cryo-extraction, which involves the pressing of frozen apples. The ice ciders made by this method are often described as more complex and aromatic, leading to the designation of "cuvée" or "special vintage" by some producers. This procedure is less used than Cryo-concentration because it is more labour intensive, more expensive and more subject to risk [12]. The making of ice ciders by the cold-pressing method requires a strict control of apple maturity in order to reach the desired content of sugars and alcoholic degree, and this is not easy to achieve. Despite this fact, the suitability of Cryo-extraction to produce ice juices and ciders in a reproducible manner has been demonstrated [13,14].

The apple cultivars or apple blends chosen for ice cider making are paramount, as they provide the final ice ciders with aromatic complexity together with high contents of sugars and malic acid to balance the flavour [9,14]. The ratio between total sugars and total acidity (TS/TA) has shown its relevance to the assessment of ice cider quality [14]. This ratio is also related to the perception of sweet taste and smooth sensation, the two attributes that most influenced the overall quality scoring of ice ciders by consumers [9]. The selection of the most suitable raw material for the making of highly-valued and differentiated ice ciders—either single-apple cultivar or apple blends—is a complex matter, as nearly 70 cider apple varieties are authorised for cider making in the Protected Designation of Origin "Cider of Asturias" (https://sidradeasturias.es/ (accessed on 8 July 2021)). A set of 10 cider apple cultivars has been assessed to evaluate their suitability for the production of ice juices. Their polyphenolic, olfactometric and sensory profiles have been described, and four varieties have been selected as potentially interesting for the making of single-cultivar ice juices [15].

The freeze-enriched apple juices are further inoculated with yeasts and fermented for several months. The technical challenges involved in the fermentation of musts with high density and high total acidity have long been highlighted. The high content of sugars present in the ice musts subjects yeasts to considerable hyperosmotic stress, leading to altered metabolism—decreased yeast growth and sugar consumption rate, production of high levels of acetic acid and glycerol—and difficulties in reaching the required alcoholic degree [16–18]. The selection of yeast strains for ice wine making takes into account their ability to ferment in high-density juices, their ethanol tolerance, nutrient requirements and hydrogen sulphide and volatile acidity production [19]. These factors have been systematically assessed in the selection of *Saccharomyces bayanus* strains for the production of ice ciders. The evaluation process concluded with the selection of 10 autochthonous yeast strains producing ice ciders with high alcoholic degree, low volatile acidity and balanced sensory profiles [20].

In addition to the above-mentioned abilities that are required to properly ferment musts with a high content of sugars, the relevance of the selected yeasts lies in their impact on the aromatic profile of the final ice products. Studies carried out on ice wines showed that different yeast species each produced a characteristic array of unique compounds or higher concentrations of specific odorants relative to each other; nevertheless, the effect of yeasts on the ice wine aroma depended upon cultivars and harvests [21,22]. The ability of three autochthonous strains of *S. bayanus* to successfully perform the alcoholic fermentation of different types of ice apple juices over two apple harvests has been demonstrated [14].

The most common yeasts for ice wine fermentation are commercial strains of *Saccharomyces cerevisiae* followed by *S. bayanus*. However, non-*Saccharomyces* strains have been

proposed in mixed fermentations to improve the aromatic complexity of ice wines [19,23]. Among the non-*Saccharomyces* yeasts, some strains of *Torulaspora delbrueckii* tolerate ethanol contents up to 8% v/v [24] and, therefore, these could also be tested for individual controlled fermentations of ice juices.

As said before, the ice ciders made by Cryo-extraction are described as more complex and aromatic; however, their production yield is quite low. Pando Bedriñana et al. have reported extraction yields of between 7.7 and 8.8 (w/w, %) for a multi-cultivar apple blend assessed over three consecutive harvests. The Exhaustion method, consisting of the freeze-enrichment of the apple juice fractions discarded from the cold-pressing system, allowed juice yield values of up to 25% to be achieved [13]. This procedure may be effective for designing new special ciders with high alcohol content and diverse aromatic profiles.

The objective of this paper is to evaluate the effect of the raw apple material and the yeast strain on the chemical and sensory characteristics of ciders made by the Exhaustion method. For this purpose, three types of cider apple juices (a single-cultivar, and blends of two and five apple varieties, referred to as J1, J2, J3) were, respectively, inoculated with three yeast strains (an autochthonous *S. bayanus* (C6), a commercial *S. cerevisiae* and a commercial non-*Saccharomyces*) to obtain a set of 18 ciders in two consecutive harvests. The physical-chemical characteristics of the enriched juices and the fermentation performance of each yeast strain were studied. The final ciders were compared on the basis of their chemical and volatile compound composition, as well as their sensory profiles.

2. Materials and Methods

2.1. Making of the Experimental Ciders

2.1.1. Enrichment of the Apple Juices

Seven cider cultivars included in the Protected Designation of Origin "Cider of Asturias" were used for the making of three types of apple juices: J1, a single variety juice made from a bitter-acidic cultivar (*Durona de Tresali*); J2, a bi-varietal juice made from a *Perico* (semi-acidic) and *Limón Montés* (acidic); J3, made up from five varieties (*Verdialona*, sweet; *Regona* and *Durona de Tresali*, bitter-acidic; *Raxao*, acidic; *de La Riega*, semi-acidic).

The cider apples were processed as described elsewhere [13]. In brief, two batches of each of the above cited mixtures (450–500 kg) were frozen at −20 °C. To produce the ice juices enriched by Cryo-extraction, the apples were pressed and the squeezed fractions were collected so as to reach a final soluble solids content of $\simeq$ 33 °Brix. The discarded apple juice fractions from each raw mixture were put together (129–207 L) and were stored in 60 L-containers, which were subsequently frozen (−20 °C) and thawed (4 °C). As the ice mass of juice thawed, successive fractions of apple juice were collected to obtain the final volume of apple must made by Exhaustion ($\approx$35 °Brix). A single batch of the enriched apple juice from each juice type was made. Then, each of these homogeneous batches were further divided into six 15-L glass containers and, respectively, inoculated with the selected yeast strains. The whole process, represented in Figure 1, was repeated in two consecutive harvests.

The concentration yield (%) at each stage or the enrichment process was calculated by means of Equation (1):

$$\text{Yield (\%)} = [C_i - C_0]/C_0 \times 100 \tag{1}$$

C_i: total soluble solids content (°Brix) in the fraction i;
C_0: total soluble solids content (°Brix) in the initial apple must.

The final extraction yields were calculated on the basis of weight of soluble solids per kg of processed apple, according to the expression (2):

$$\text{Yield (\%)} = [V \times D]/W \times 100 \tag{2}$$

V: Volume of enriched apple juice (L).
D: Density of the enriched apple juice (kg/L).
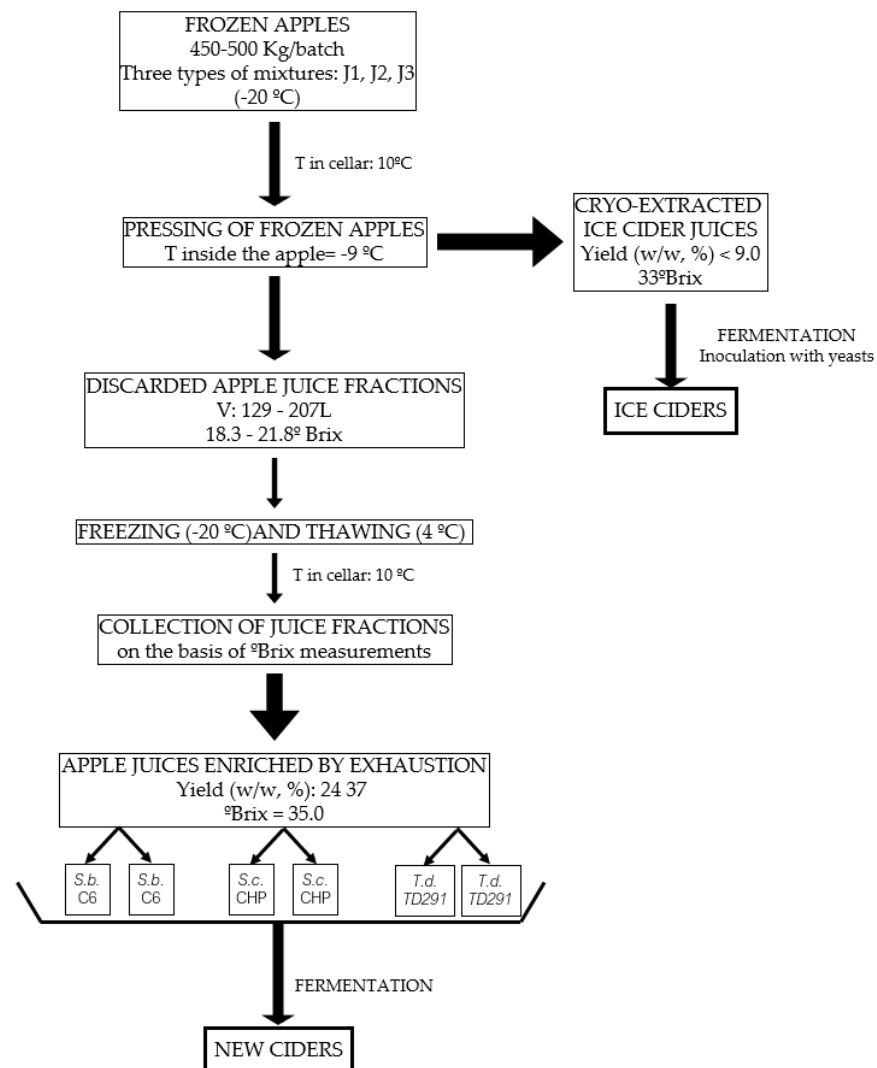W: Weight of processed apples (kg).

Figure 1. Scheme of the making of the new ciders obtained by the Exhaustion method. J1: *Durona de Tresali*; J2: *Perico + Limón Montés*; J3: *Verdialona + Regona + Durona de Tresali + de la Riega + Raxao*; S.b.: *Saccharomyces bayanus* C6; S.c.: *Saccharomyces cerevisiae* CHP; T.d.: *Torulaspora delbrueckii* TD291.

2.1.2. Fermentation of the Enriched Apple Juices

Three yeast strains were used: the autochthonous *S. bayanus* strain (C6) and two commercial yeasts: *S. cerevisiae* strain (Levuline-CHP) and *T. delbrueckii* strain (Biodiva-TD291), both provided by Lallemand.

The required amount of the indigenous yeast strain was obtained by fed-batch cultivation in 2 L-stirred tank bioreactors (Biostat-B plus, Sartorius, Göttingen, Germany) at 30 °C for 22 h, as described elsewhere [14]. Subsequently, the yeasts were concentrated by filtration (Sartoflow Slice 200, Sartorius Göttingen, Germany) to 0.15 L and stored at 4 °C until use as inoculum (yeast cream). The juices were inoculated with 0.66 g/L of the indigenous yeast cream (10×10^9 cfu/mL) and the commercial strains according to supplier's recommendations (0.25 g/L).

All the yeast strains were dissolved in a volume equivalent to 10 times their weight of water containing 0.3 g/L of the nutrient GO-FERM (Lallemand, Montreal, Canada) following a stepwise acclimatization. All the experimental fermentations were done in duplicate at 15 °C in 15 L-glass containers (18 ciders each harvest). The fermentation processes were monitored by periodically taking samples for the counting of microorganisms, and the measurements of the corresponding densities and alcoholic degrees. After fermentation, the ciders were racked-off, clarified, stabilised and bottled as described elsewhere [13].

2.2. Analytical Procedures

2.2.1. Microbiological Analysis

Samples of the enriched musts and ciders were taken aseptically at different stages of the cider-making process. Several 1/10 (*v/v*) dilutions were done with a Ringer's solution, spread by triplicate onto different agar media (WL Nutrient Agar (Microkit, Madrid, Spain), ZMA medium and basal medium), prepared and incubated according to the conditions described elsewhere to, respectively, count the populations of yeasts, lactic acid and acetic bacteria [13].

2.2.2. Oenological Parameters

Freeze-enriched juices and ciders were analysed for oenological parameters. Density, total acidity at pH = 8.20, total phenols (Folin–Ciocalteu method) and pH were determined according to international normalised methods [25,26]. Sugars and polyalcohols (sucrose, glucose, fructose and sorbitol), as well as organic acids (malic, lactic and shikimic) were determined by HPLC as reported elsewhere [27,28]. The ciders were also analysed for volatile acidity and glycerol contents.

2.2.3. Volatile Compounds

Bottled ciders were analysed for volatile compound profiles.

1. Major volatiles (ethyl acetate, methanol, 1-propanol, *iso*-butanol, 1-butanol, amyl alcohols, 2-phenylethanol) were analysed by GC-FID in the split mode (1/5) previous distillation of the sample by direct heating, and were eluted with helium (1 mL/min) according to the conditions reported elsewhere [28].
2. Minor volatiles (alcohols, esters, fatty acids and volatile phenols) were determined by Stir Bar Sorptive Extraction and Gas Chromatography with Mass Spectroscopy (SBSE-GC-MS) using a 7890 GC coupled to a 5975 MSD (Agilent, Palo Alto, CA, USA) as described previously [14]. Briefly, the volatiles were adsorbed onto a polydimethylsiloxane coated bar (20 mm length, 0.50 mm thickness phase, Twister, Gerstel GmbH & Co, Mülheim und der Ruhr, Germany) suspended in the headspace of a 60 mL-vial and stirred at 700 rpm for 18 h, at 20 °C. The volatile profiles were quantified in SIM mode and the results expressed as µg of internal standard (2-ethyl-1-hexanol, 1.17 mg/L) per liter.

2.3. Sensory Analysis

The ciders were assessed each harvest at 10–12 °C in normalised glasses (ISO 3591:1977) by nine people belonging to the Sensory Committee of the Protected Origin Designation "Cider of Asturias" in three sessions and in a sequential monadic presentation, as reported previously [13]. Six odour/aroma attributes (candy, apple-like, fruity, floral, butter and mushroom) and four taste/mouthfeel attributes (sweet, acidic, bitter and astringent) are identified by the sensory panel and analysed as citation frequencies. Scorings for overall quality were obtained by using a 5-point discontinuous scale (5, excellent; 4, good; 3, fair; 2,; poor; 1, defective).

2.4. Statistical Analyses

The R-platform (R-commander program version 2.7-1 for Windows) was used throughout the study. The Shapiro–Wilks test was first used to evaluate the normality of data.

Non-parametric tests (Wilcoxon and Kruskal–Wallis) were used to evaluate the influence of the type of juice and harvest on the chemical composition of the enriched-apple juices. The same statistical tests were used to assess the effect of the above-mentioned factors and the yeast strain on the chemical composition, the yeasts' fermentative performances and the sensory attributes of the ciders.

Principal Component Analysis (18 objects and 16 variables) was performed to ascertain the relationships between samples and chemical and sensory variables.

3. Results

3.1. Enrichment of the Apple Musts

The apple cultivars were chosen on the basis of their chemical and sensory characteristics and their maturation dates. All these varieties are of late maturation. Three different types of apple juice have been made: the mono-cultivar *Durona de Tresali* (J1), characterised by its sensory quality; the bi-cultivar blend (J2), made up of *Perico* and *Limón Montés*, having complex aromatic profiles [15]; the multi-cultivar apple blend (J3), consisting of a mixture of five varieties (*Verdialona*, *Regona*, *Durona de Tresali*, *Raxao* and *de la Riega*), providing the typical mild-sharp profile of the Asturian cider.

The extraction of freeze-enriched juices is characterised by its low yield [29]. Ice cider is a special and expensive beverage requiring 3–4.5 kg of apples to produce a small 375-mL bottle. The production process is time-consuming and technically demanding, from the selection of the most suitable cider apple varieties to the strict control of cellar temperatures during pressing.

The ice apple juices obtained by Cryo-extraction were made by pressing the corresponding batches of frozen cider apple cultivars or blends at a cellar temperature around 10 °C. This temperature should be low enough to allow the obtention of the enriched juice before the water inside the apple tissue thaws [30]. In these trials, once the pressing of around 500 kg of apples had started, only the juice obtained in the first 4–4.5 h, with density values equal to or greater than 1.1400 g/mL, was suitable for making ice ciders with the minimum characteristics required (alcoholic degree, 8% *v/v*, residual sugar content of 100 g/L) by the Spanish Legislation [11]. The volumes of the collected cryo-extracted apple juices ranged between 27.8 and 37.4 L, the extraction yields being lower than 9% (*w/w*).

After these first hours of pressing, the apples were still frozen in the press and therefore, by increasing the pressing time, it was possible to obtain apple juices with mean total soluble solid values of 16.2–23.8 °Brix (density values around 1.0651–1.0988 g/mL), which are higher than those normally achieved by pressing unfrozen apples [31]. In the working conditions of the current study, the flow of squeezed juice ranged between 3–4 L/h. The final volumes of the apple juices discarded from the Cryo-extraction processes ranged between 129 and 207 L, depending on the juice type. These musts were distributed in 60-L containers and frozen at −20 °C to later carry out the Exhaustion process.

This second freeze-enrichment procedure starts by thawing the apple musts at 4 °C, and successively collecting fractions of decreasing soluble solids content. Figure 2a shows the changes in total soluble solids values of the different fractions collected during the processing of three containers of the apple juice referred to as J3. The whole exhaustion process was extended to 48 h. Figure 2b shows the concentration yield of the apple musts (%), calculated according to expression (1), and the total soluble solids of the enriched musts during the process. The highest yields (61.6% ± 6.1) were reached after approximately 8 h of work, the total solids concentration being 52.0 ± 0.6 °Brix. From this point on, and until the end of the Exhaustion process, both the performance of the operation and the Brix degree of the resulting enriched apple musts decreased. The concentration yield drastically dropped after 26 h of work. A final fraction was taken at 48 h both to complete the desired volume of must and to standardise the initial values of total soluble solids to start the fermentation around 35 °Brix.

The yield (%) of the Exhaustion process with regard to the weight of apples pressed, calculated from Equation (2), ranged between the 24% achieved with the multicultivar blend J3 and the 37% obtained with the apples referred to as J1 and J2, independently of the harvest.
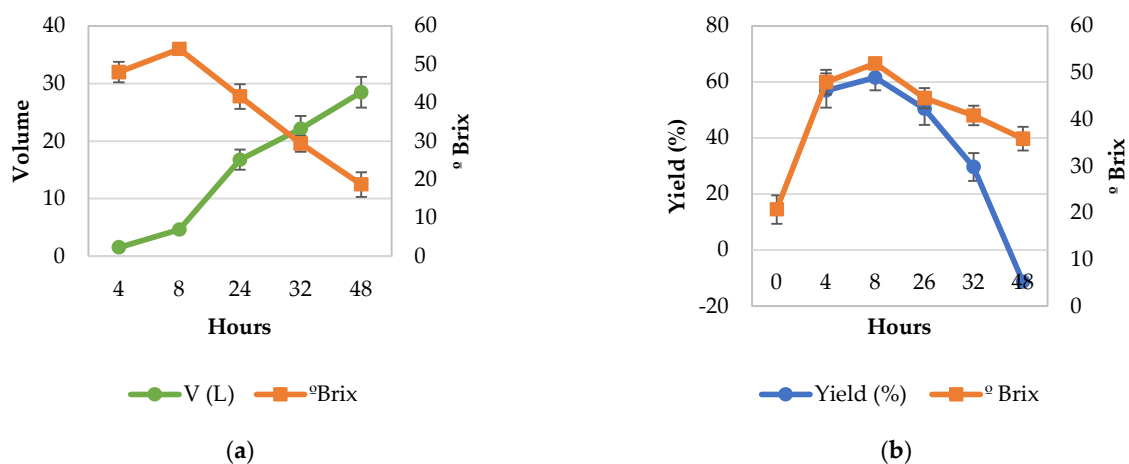
(**a**)

(**b**)

Figure 2. (**a**): Variations in total soluble solids content in the apple juices during the Exhaustion process; (**b**) changes in yield (%) and soluble solids concentration of the different enrichment apple must fractions throughout the Exhaustion process. Data of volume and °Brix represented are mean (±SD) of three 60-L containers of the multi-cultivar juice (J3).

3.2. Microbiological and Chemical Characteristics of the Enriched Juices

Yeasts, ranging between 7.80×10^3 and 6.75×10^5 cfu/mL, were the most abundant microorganisms in all the juices, their concentrations being similar to those reported previously for cryo-extracted ice juices [14]. All the colonies had the same shape and colour (red-brown at the bottom), and pigmented the WL Nutrient Agar. This description is consistent with that reported by Pallman et al. [32] for *Metschnikowia pulcherrima*, and coincident with the species isolated and identified by Pando Bedriñana et al. in freeze-enriched apple juices [13]. Yeasts of this type isolated from cider exhibit interesting protease and β-glucosidase properties, which are important for aroma improvement [33]. Moreover, members of the *Metschnikowia* genus have been detected in ice wines produced in different geographical areas, such as Northern Italy [34], Canada [35] and Slovakia [36]. Strains of this yeast have also been used in simultaneous and sequential fermentation trials with *S. cerevisiae* to improve aroma diversity of Vidal blanc ice wine [23].

Acetic acid bacteria were the second most important group, with a maximum level of 1.60×10^4 cfu/mL in the juice referred to as J2 in the second harvest. These microorganisms are strictly aerobic and, therefore, their development is limited once the alcoholic fermentation has started. Lactic acid bacteria were detected in two of the six experimental juices (J1, J2 in the second harvest), their concentrations being lower than 305 cfu/mL. The low pH of the enriched apple juices, ranging between 3.22 (J3, first harvest) and 3.43 (J1, second harvest), may explain, in general, these data. This parameter was significantly influenced by the juice-type ($p < 0.05$). It is worth noting that none of these bacterial groups were further detected during the fermentation process (data not shown).

Total acidity ranged between 12.07 and 15.59 g sulphuric acid/L. The acidity values were nearly three-fold those observed in single-cultivar juices of Spanish cider apple varieties [37,38], and significantly lower in the juice referred to as J1 ($p < 0.10$). Total phenols, ranging between 4.0 and 4.4 g tannic acid/L, also experienced increases in their levels equivalent to three-fold those found in the conventional pressed apple juices [37]. Malic acid was the main acid found in all the juices. Its content varied between 15.5 (J1) and 20.3 (J3). The shikimic acid content ranged between 26 and 63 mg/L in the first harvest, and between 39 and 56 mg/L in the second harvest. The juice referred to as J2 presented the highest levels, and the mono-cultivar juice, the lowest.

The Exhaustion method allowed the final density of the enriched-apple juices to be accurately standardised between 1.1478–1.1490 g/mL.

The contents of total sugars in the freeze-enriched apple juices were similar to those previously reported in the juice obtained by Cryo-extraction [14] and significantly higher

in the juices made during the second harvest ($p < 0.10$). Fructose, the main sugar in apple, ranged between 148.2 g/L (J2, first harvest) and 201.9 g/L (J3, 2nd harvest). The second sugar component was sucrose or glucose, depending on the cider apple raw material (Figure 3). The mean contents observed in these freeze-enriched apple juices were more than three-fold those reported for conventional musts of cider or juice apple cultivars. Similar concentration factors were obtained for sorbitol, whose contents ranged between 18.1 and 26.8 g/L in the enriched apple juices [39,40]. This alcohol has been identified as a significant contributor to the perception of the sweet taste of apples [41]; it has been also associated with the smoothness mouthfeel and, consequently, with the quality assessment of ice ciders made by Cryo-extraction [9].
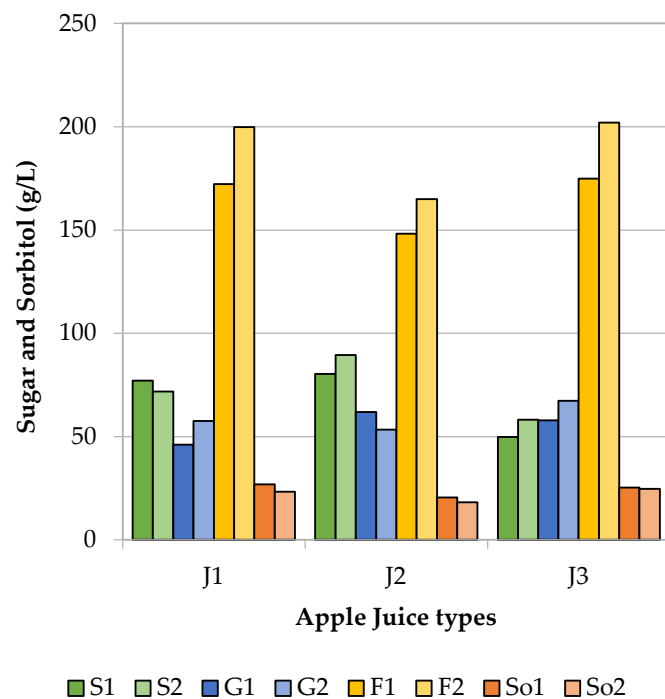

Figure 3. Sugars and sorbitol profiles of the freeze-enriched apple juices: Sucrose (S), Glucose (G), Fructose (F) and Sorbitol (So). J1: *Durona de Tresali*; J2: *Perico + Limón Montés*; J3: *Verdialona + Regona + Durona de Tresali + Raxao + de la Riega*. Dark colours, harvest 1; Light colours, harvest 2.

It is worth noting that both the Exhaustion process described in the current study and the Cryo-extraction process previously reported [14] maintained, in the respective freeze-enriched musts, the relative proportions of sugars and sorbitol observed in conventional apple juices. This is a common feature to other freezing juice concentration processes, as the distribution of solutes between the liquid and ice phases is not selective [42].

3.3. Fermentation Behaviour of Pure Cultures

Six subsets (15 L) of the above obtained exhaustion apple juices were made, and were subsequently inoculated with the strains *S.b.* C6, *S.c.* CHP and *T.d.* TD291. The fermentation process was monitored by taking samples every 15 days for the counting of microorganisms and the measurement of the corresponding densities and alcoholic degrees. The fermentation process was ended when an alcoholic degree of 9 (% *v/v*) was reached.

In Table 1 are summarised the main fermentation performances of each yeast strain at 15 days of fermentation, as well as the chemical characteristics of ciders. The Biodiva-TD291 yeast strain showed significantly slower fermentation rates ($p < 0.01$), requiring between 75 (first harvest) and 105 days (second harvest) to reach an alcoholic degree higher than 9 (% *v/v*). A very different rate of fermentation was observed in the *S. cerevisiae* strain, which provided the ciders with the highest alcoholic degrees in just 30 days, in both

harvests. The autochthonous C6 yeast strain exhibited different fermentation rates, slower in the second harvest (60–75 days) than in the first harvest (20–30 days).

The maximum yeast concentration was reached at 15 days of fermentation in all cases. At this point, the maximum ethanol production rate (g/L/day) was also obtained, with a significantly lower value of this parameter being observed in the case of the *T. delbrueckii* yeast strain. In this sense, lower ethanol production yields for *T. delbrueckii* species have also been observed in fermentations of high-sugar musts of botrytised grapes [43]. Subsequently, only the *S. cerevisiae* strain was able to continue producing ethanol at a rate of 2.37 g/L/day (data not shown) and, as a consequence, the ciders made with this yeast strain reached higher alcoholic degrees ($p < 0.01$). The final fermentation yield (g ethanol/g sugar consumed) was quite similar, the influence of the apple juice type and the yeast strain not being significant ($p > 0.10$).

The production of glycerol and acetic acid is the osmorregulatory mechanism reported in *S. cerevisiae* to cope with osmotic strength in high-sugar media. Unlike the C6 yeast strain, the Levuline-CHP yeast strain generated the highest-level values of volatile acidity ($p < 0.01$), which reinforce the utility of selected autochthonous yeast strains to control the final acetic content in this type of difficult fermentation [13,20]. The *T. delbrueckii* species is also a low acetic acid producer compared to most of the non-*Saccharomyces* yeasts [44]. In the second harvest, this yeast strain provided ciders with the lowest values of volatile acidity (0.43 ± 0.04 g acetic acid/L).

The glycerol content ranged between the lower levels observed in the ciders made with the *T. delbrueckii* yeast strain and the higher contents produced by the autochthonous *S. bayanus* yeast strain referred to as C6. These values were two- or three-fold those reported in conventional ciders [28,45], the effect of harvest and yeast strain being significant (Table 1). On the one hand, during the first harvest, the C6 yeast strain gave a higher glycerol content (8.3–9.6 g/L) in ciders than in those of the second harvest (6.9–7.1 g/L), coinciding with a faster fermentation rate. On the other hand, the TD291 yeast strain yielded the lowest glycerol concentrations in the ciders made in the second harvest (5.3–5.7 g/L), which confirms that this species is highly resistant to stress conditions [43].

Sorbitol was the main polyalcohol observed in the ciders. Its content, ranging between 16.4 and 27.0 g/L, was significantly influenced ($p < 0.01$) by both the apple juice type and the harvest.

The three yeast strains consumed part of the malic acid present in the initial juice. Average decreases in the contents of malic acid in ciders were 18%, 13% and 14%, respectively, for yeast strains referred to as C6, CHP and TD291. These results were in agreement with those reported elsewhere, which confirmed that malic acid can be metabolised by several yeast species, leading to reductions of up to 20% [44]. It has also been reported that *S. bayanus* species possess the capacity to degrade malic acid, [46]. In the conditions of the current study, the contents of malic acid in ciders were significantly influenced by the type of apple juice and harvest (Table 1). Taking into account that this acid represented more than 95% of the total acidity of these ciders, the decreased content observed in the final ciders exerts an important effect on the respective total sugars/total acidity ratios (TS/TA). As shown in Table 1, the ciders made from the single-cultivar apple juice (J1) reached the highest TS/TA ratio values ($p < 0.01$).

Table 1. Fermentation behaviour of pure cultures and chemical characteristics of ciders according to the apple juice-type, yeast strain and ranges of variation in each harvest (minimum-maximum).

	Juice Type						Yeast Strains			Harvests	
	J	S	H	J1	J2	J3	C6	CHP	TD291	1	2
15 days fermentation											
Maximum yeast concentration (log cfu/mL)	ns	***	**	7.74 ± 0.42	7.79 ± 0.77	8.01 ± 0.97	8.43 ± 0.85	7.56 ± 0.39	7.54 ± 0.55	6.81–8.14	7.15–9.85
Maximum ethanol production rate (g/L/day)	ns	***	ns	3.57 ± 1.09	3.31 ± 1.23	3.40 ± 1.15	4.23 ± 0.80	4.00 ± 0.42	2.05 ± 0.35	1.65–5.06	2.08–4.35
Final ciders											
Ethanol yield (g ethanol/g sugar consumed)	ns	ns	***	0.46 ± 0.05	0.49 ± 0.04	0.46 ± 0.07	0.45 ± 0.06	0.49 ± 0.05	0.46 ± 0.06	0.49–0.54	0.37–0.49
Total sugars (g/L)	ns	***	***	122.6 ± 9.3	127.1 ± 9.0	123.5 ± 7.0	128.4 ± 8.6	115.7 ± 3.3	129.2 ± 4.4	112.1–135.2	111.5–142.8
Alcoholic degree (% *v/v*)	ns	***	**	10.89 ± 0.93	10.57 ± 0.97	10.43 ± 0.83	10.02 ± 0.74	11.69 ± 0.29	10.20 ± 0.39	10.10–12.00	9.20–12.10
Total acidity (sulphuric acid/L)	***	ns	ns	11.70 ± 0.32	13.12 ± 1.04	13.89 ± 1.41	12.54 ± 1.17	12.98 ±1.61	13.19 ± 1.29	9.65–14.59	11.48–15.09
Volatile acidity (g acetic acid/L)	ns	***	ns	0.65 ± 0.15	0.58 ± 0.12	0.64 ± 0.15	0.54 ± 0.10	0.75 ± 0.05	0.58 ± 0.15	0.43–0.80	0.41–0.81
Total phenols (g tannic acid/L)	***	ns	ns	3.5 ± 0.1	4.0 ± 0.3	3.7 ± 0.2	3.6 ± 0.2	3.8 ± 0.3	3.7 ± 0.3	3.4–4.4	3.4–3.9
Glycerol (g/L)	ns	**	***	7.7 ± 1.3	7.2 ± 1.0	7.4 ± 0.9	7.9 ± 1.0	7.8 ± 0.4	6.6 ± 1.2	7.3–9.6	5.1–7.8
Sorbitol (g/L)	***	ns	***	23.0 ± 3.3	18.7 ± 2.1	23.5 ± 3.2	21.8 ± 3.8	21.7 ± 3.7	21.6 ± 3.5	20.3–27.0	16.4–20.6
Total sugars/Total acidity	***	ns	ns	10.5 ± 0.9	9.8 ± 1.3	9.0 ± 1.1	10.3 ± 1.2	9.0 ± 1.2	9.9 ± 1.0	7.8–11.9	7.7–12.3
Total sugars/Total phenols	ns	***	**	35.1 ± 3.4	32.3 ± 4.0	33.5 ± 2.8	35.5 ± 3.3	30.3 ± 2.3	35.2 ± 2.2	25.7–37.4	29.6–41.8
Malic acid (g/L)	***	ns	***	15.8 ± 0.7	17.2 ± 2.2	19.1 ± 0.9	16.8 ± 1.9	17.8 ± 2.0	17.5 ± 2.0	15.5–20.3	14.6–18.8
Shikimic acid (mg/L)	***	***	ns	39.0 ± 12.8	67.1 ± 11.5	58.2 ± 10.9	68.0 ± 12.4	47.4 ± 14.1	48.8 ± 14.9	23.4–86.8	39.4–75.2

J: juice; S: strain; H: harvest; J1: *Durona de Tresali*; J2: *Perico + Limón Montés*; J3: *Verdialona + Regona + Durona de Tresali + de la Riega +Raxao*; Total Sugars: Sucrose + Glucose + Fructose; ns: not significant; (**) significant at $p < 0.05$; (***) significant at $p < 0.01$.

The contents of shikimic acid ranged between 23 and 87 mg/L, depending on the initial apple juice and the yeast strain involved in the fermentation (Table 1). The ciders made from the single-cultivar apple juice (J1) reached the lowest contents of shikimic acid, whereas the yeast strain referred to as C6 provided ciders with the highest levels of this acid, in particular with the juice referred to as J2 in the first harvest ($p < 0.01$). It has been reported that the ability to produce higher contents of shikimic acid is a typical trait of cryo-tolerant strains, such as *S. bayanus*, against *S. cerevisiae* [44]. Shikimic acid can be a precursor to some volatile compounds, such as ethyl cinnamate and benzaldehyde. This organic acid is also involved in the synthesis of the aminoacid phenylalanine, which is the precursor of 2-phenylethanol and 2-phenyl ethyl acetate, two odorants identified as important contributors to the aromatic structure of cider [47].

3.4. Volatile Compounds of Ciders

The analysis of the volatiles profiles of the ciders led to the quantification of 38 compounds, which are summarised in Table 2.

Higher alcohols (propanol, iso-butanol, amyl alcohols and 2-phenylethanol) were quantitatively the largest group of flavour compounds, their contents being in the ranges usually observed in many other types of cider [45,48]. They are secondary by-products of the alcoholic fermentation, produced by yeasts through conversion of branched chain aminoacids and degradation of the sugars. The metabolism of the aminoacids to higher alcohols takes place during the exponential growth phase, and part of the enzymatic pathways involved in the synthesis of aminoacids are used in the formation of higher alcohols from sugars [49]. As shown in Table 2, the contents of these volatile compounds were significantly influenced by the yeast strain. The yeast named as Biodiva-TD291 gave the lowest content of amyl alcohols, in agreement with results reported elsewhere, identifying *T. delbrueckii* species as low producers of fusel alcohols [44]. On the contrary, the autochthonous C6 provided the ciders with the highest content of amyl alcohols. The ciders made with this yeast strain in the first harvest reached concentration values of amyl alcohols higher than 300 mg/L, coinciding with a faster fermentation rate and a higher rate of ethanol production per day (ranging between 4.92 and 5.06 g/L/day). Likewise, the yeast referred to as C6 provided the ciders with the highest levels of 2-phenylethanol; in particular, those made with the bi-varietal blend (J2) in the first harvest (149.8 ± 7.1 mg/L). The production of this rose-scented or honey-like odorant is a typical characteristic of *S. bayanus* with respect to the other two species investigated [14,46].

The group of esters was qualitatively the most numerous. Ethyl acetate was the main component, its content being significantly influenced by the yeast strain. The autochthonous *S. bayanus* provided ciders with the lowest concentrations. The rest of the esters (23 compounds) were analysed as minor volatiles by SBSE. Four medium-chain fatty acid ethyl esters (decanoate, octanoate, hexanoate and dodecanoate) constituted between 67 and 83% of the minor volatile composition. The non-*Saccharomyces T. delbrueckii* yeast strain provided ciders with the lowest ethyl octanoate contents whereas the yeast strain named as CHP yielded the highest concentration of ethyl hexanoate. The influence of the apple juice type was significant on the ethyl decanoate contents, with the ciders made from the bi-varietal blend exhibiting the highest levels. Many minor esters were also influenced by the harvest factor (Table 2).

The acetate esters represented up to 11% of the minor volatile composition. Isoamyl acetate and 2-phenylethyl acetate were the main representatives of this family. Similar to the results described above regarding the levels of 2-phenylethanol, the yeast referred to as C6 yielded the highest contents of 2-phenylethyl acetate, particularly in the ciders made from the bi-varietal blend in the first harvest (34.4 ± 0.6 µg IS/L) (Table 2).

Finally, four fatty acids were quantified; among these, decanoic and octanoic acids constituted between 8 and 21% of the minor volatile composition. Their contents were significantly higher in the ciders made during the second harvest.

Table 2. Volatile compounds composition of ciders obtained by Exhaustion according to the apple juice-type, yeast strain, and ranges of variation in each harvest (Minimum-Maximum).

		Juice Type					Yeast Strains			Harvests	
	J	S	H	J1	J2	J3	C6	CHP	TD291	1	2
Major volatiles (mg/L)											
Ethyl acetate	ns	***	ns	57.5 ± 12.4	61.2 ± 13.3	62.9 ± 12.4	50.4 ± 5.7	69.8 ± 7.0	61.4 ± 14.7	42.0–80.2	44.1–78.8
Methanol	***	ns	***	46.1 ± 6.1	48.5 ± 5.4	34.9 ± 6.5	43.8 ± 8.5	42.9 ± 8.2	42.7 ± 9.1	39.8–54.6	27.1–43.8
1-Propanol	ns	***	*	36.3 ± 12.5	33.3 ± 11.9	33.8 ± 9.2	20.9 ± 3.6	44.9 ± 4.4	37.6 ± 5.2	21.8–51.5	16.2–50.7
Iso-butanol	ns	***	***	33.7 ± 8.2	36.7 ± 8.9	33.9 ± 8.7	37.2 ± 8.4	26.9 ± 1.1	40.3 ± 7.1	25.0–50.7	25.7–36.3
1-butanol	***	ns	*	3.4 ± 0.1	3.1 ± 0.2	5.9 ± 0.4	4.0 ± 1.3	4.1 ± 1.3	4.3 ± 1.4	2.7–5.8	3.1–6.6
Amyl alcohols	ns	***	ns	218.4 ± 43.9	220.1 ± 41.4	224.2 ± 45.5	267.2 ± 44.2	203.1 ± 4.6	191.8 ± 8.9	188.3–317.4	180.6–236.2
2-Phenylethanol	ns	***	ns	34.9 ± 23.4	57.5 ± 46.6	32.8± 18.3	77.6 ± 34.2	23.3 ± 10.2	24.2 ± 8.7	15.3–154.9	18.0–69.3
Minor volatiles (μg/L I.S.)											
Minor alcohols + Volatile phenols											
Hexanol	ns	ns	***	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5–0.7	0.4–0.6
1-Octanol	***	***	***	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1–0.2	0.1–0.3
4-vinylguaiacol	**	***	ns	0.9 ± 0.6	0.7 ± 0.5	0.3 ± 0.2	1.2 ± 0.6	0.5 ± 0.2	0.3 ± 0.1	0.2–1.9	0.2–2.0
4-vinylphenol	*	***	ns	0.4 ± 0.3	0.2 ± 0.2	0.1 ± 0.1	0.5 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.1–1.0	0.1–0.6
Esters											
Ethyl butyrate	ns	ns	***	1.2 ± 0.7	0.9 ± 0.4	1.1 ± 0.5	1.0 ± 0.5	1.2 ± 0.6	0.9 ± 0.9	0.4–1.0	1.0–2.5
Ethyl-2-methylbutyrate	ns	**	***	1.1 ± 0.9	0.8 ± 0.3	1.0 ± 0.5	1.3 ± 0.7	0.9 ± 0.5	0.7 ± 0.5	0.2–1.4	0.6–2.9
Ethyl-3-methylbutyrate	ns	***	**	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1–0.4	0.1–0.2
Ethyl valerate	**	ns	***	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1–0.2	0.1–0.4
Ethyl hexanoate	ns	*	*	20.6 ± 12.9	15.9 ± 5.8	15.5 ± 8.5	16.3 ± 6.7	22.3 ± 12.7	13.5 ± 6.3	6.0–37.6	8.4–54.1
Ethyl octanoate	ns	***	ns	139.7 ± 61.0	151.2 ± 47.8	119.2 ± 45.1	151.2 ± 52.8	171.7 ± 33.7	87.3 ± 21.4	62.4–224.3	59.1–239.3
Ethyl nonanoate	ns	ns	***	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1–0.3	0.2–0.4
Ethyl decanoate	*	ns	ns	167.2 ± 35.6	190.5 ± 48.3	144.5 ± 40.4	155.3 ± 51.9	187.6 ± 25.2	159.2 ± 48.7	61.9–267.3	112.9–218.6

Table 2. *Cont.*

	J	S	H	J1	J2	J3	C6	CHP	TD291	1	2
				Juice Type			**Yeast Strains**			**Harvests**	
Ethyl benzoate	***	ns	***	4.1 ± 1.1	2.6 ± 1.1	2.5 ± 0.4	3.5 ± 1.3	2.6 ± 0.9	3.1 ± 1.1	1.4–4.1	2.3–5.6
Diethyl succinate	ns	***	***	2.5 ± 1.3	3.2 ± 1.7	3.2 ± 1.0	4.1 ± 1.5	2.5 ± 1.0	2.2 ± 0.7	1.1–3.7	2.0–7.0
Ethyl dodecanoate	ns	ns	*	22.1 ± 9.3	20.1 ± 8.4	16.5 ± 12.0	19.8 ± 8.1	22.7 ± 12.4	16.1 ± 8.7	4.9–29.7	9.4–41.6
Ethyl tetradecanoate	ns	**	**	4.4 ± 3.6	3.3 ± 2.0	3.7 ± 3.9	4.9 ± 2.4	3.8 ± 3.5	2.7 ± 3.5	0.5–9.5	0.9–12.6
Ethyl cinnamate	*	ns	***	1.0 ± 0.5	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.4	0.6 ± 0.2	0.7 ± 0.4	0.4–0.9	0.5–1.7
Ethyl hexadecanoate	ns	**	ns	5.4 ± 2.6	5.5 ± 1.9	6.5 ± 3.2	7.1 ± 1.6	4.7 ± 2.6	5.6 ± 3.0	1.9–8.2	2.5–12.3
Ethyl oleate	ns	***	ns	0.6 ± 0.4	0.9 ± 0.9	0.7 ± 0.9	1.3 ± 1.1	0.6 ± 0.3	0.3 ± 0.1	0.1–3.2	0.2–1.0
Isoamyl decanoate	ns	ns	**	1.2 ± 0.3	1.4 ± 0.4	1.2 ± 0.4	1.4 ± 0.4	1.3 ± 0.2	1.1 ± 0.4	0.7–2.0	0.9–2.0
Isoamyl octanoate	ns	***	**	1.6 ± 0.9	2.2 ± 1.0	1.5 ± 0.7	2.4 ± 1.1	2.0 ± 0.4	1.0 ± 0.3	0.6–2.2	0.8–4.1
Methyl octanoate	***	ns	***	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1–0.2	0.1–0.5
Methyl decanoate	***	ns	ns	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.1–0.3	0.1–0.3
Isoamylethyl acetate	ns	***	**	0.8 ± 0.4	1.0 ± 0.4	1.0 ± 0.4	1.4 ± 0.1	0.7 ± 0.3	0.7 ± 0.3	0.3–1.5	0.6–1.7
Ethylphenyl acetate	***	ns	***	1.3 ± 0.3	2.6 ± 0.4	2.0 ± 0.6	2.1 ± 0.6	1.8 ± 0.6	2.0 ± 0.8	0.9–2.9	1.3–3.3
Isoamyl acetate	ns	ns	ns	9.7 ± 3.6	10.7 ± 3.8	9.5 ± 3.2	9.5 ± 2.4	9.3 ± 3.6	11.1 ± 4.1	4.8–17.9	5.0–17.0
2-Phenylethyl acetate	ns	ns	***	4.0 ± 2.7	10.7 ± 12.4	3.8 ± 1.8	9.7 ± 11.7	4.7 ± 5.4	4.0 ± 3.2	3.4–34.8	0.7–4.2
Fatty acids											
Hexanoic	ns	ns	***	1.6 ± 0.7	1.6 ± 0.7	1.5 ± 0.7	1.4 ± 0.8	2.0 ± 0.7	1.3 ± 0.3	0.6–1.6	1.5–3.0
Octanoic	ns	*	***	23.3 ± 12.7	24.7 ± 15.8	22.1 ± 11.6	28.9 ± 18.2	27.0 ± 8.1	14.1 ± 3.1	9.7–24.5	13.3–55.2
Decanoic	ns	ns	***	41.1 ± 13.5	41.2 ± 13.7	38.2 ± 16.7	39.6 ± 18.6	43.8 ± 13.0	37.1 ± 10.6	16.1–42.9	32.6–64.9
Dodecanoic	ns	ns	***	1.7 ± 0.5	1.7 ± 0.7	1.6 ± 1.3	1.6 ± 0.5	1.8 ± 1.0	1.6 ± 1.1	0.4–2.3	0.9–4.4

J: juice; S: strain; H: harvest; J1: *Durona de Tresali*; J2: *Perico + Limón Montés*; J3: *Verdialona + Regona + Durona de Tresali + de la Riega +Raxao*; Total Sugars: Sucrose + Glucose + Fructose; ns: not significant; (*) significant at $p < 0.10$; (**) significant at $p < 0.05$; (***) significant at $p < 0.01$.

The effect of the system for obtaining the freeze-enriched juices (Exhaustion vs. Cryo-extraction) on the aromatic composition of the ciders was evaluated with the ciders made by fermentation with the *S. bayanus* yeast strain (Table S1). Regarding the major volatile profiles, it is worth noting that the Cryo-extraction system provided ciders with a higher 2-phenylethanol content ($p < 0.01$).

Among the minor volatile components, the general trend observed was that the concentrations of all odorants in the present ciders, made by Exhaustion, were significantly lower ($p < 0.01$) than those found in the ice ciders obtained by Cryo-extraction [14]. This feature was especially noticeable in the case of certain odorants, such as ethyl tetradecanoate and isoamyl acetate, whose contents in the ice ciders were, respectively, 10 and 4.8 times higher than those obtained in the ciders obtained by Exhaustion. Similarly, the concentrations of ethyl decanoate and ethyl octanoate in the present ciders were half of those observed in the ciders made by Cryo-extraction. The synthesis of acetate esters is regulated by the concentration of their substrates (acetyl-CoA and fusel alcohols) and the total activity of the enzymes involved in the formation of the respective esters. Specific characteristics of the fermentation medium, such as sugar contents, lipid concentration and dissolved oxygen, influence the final amounts of acetate esters. In the case of the production of ethyl esters, the substrate concentration (fatty acids and ethanol) and the balance between synthetic and hydrolytic enzymatic activities are important mechanisms, influencing their contents in the fermented beverages [50]. In this sense, a more intense aeration of the apple juices during the freeze-enrichment of the musts obtained by Exhaustion, and a lower concentration of aromatic substrates in the discarded fractions of the Cryo-extraction process, could explain the differences in the volatile profiles of the two types of cider.

3.5. Sensory Profiles

The judges identified five aromatic attributes (candy, fruity, apple-like, floral and butter), together with four taste and tactile descriptors (sweet, acidic, bitter and astringent). The overall quality of ciders was influenced by the yeast strain ($p < 0.10$), the ciders made with the yeast strain *S.c.* Levuline-CHP being the least appreciated. In the first harvest, only the ciders made from the single cultivar (J1) scored as fair, whereas, in the second, harvest the fermentation of this juice with the yeast strains *S.b.* C6 and *T.d.* TD291 provided the ciders with the highest overall quality. The ciders obtained from the multicultivar apple juice (J3) were described as fruitier, while the juice referred to as J1 provided the ciders with the highest citation values for the apple attribute, and the attribute butter was predominant in the ciders made from the bi-varietal blend (J2). The non-*Saccharomyces* yeast strain gave the most balanced and fresh ciders, in particular, those made from the juice referred to as J3. The most bitter, astringent and buttery-scented ciders were those obtained in the first harvest (Table 3).

A Principal Component Analysis was performed to identify possible relationships among ciders (18), using eight sensory attributes (candy, apple, floral, acidic/fresh, butter, bitter, astringent and overall quality) and eight chemical variables, namely: ratios between Total sugars and Total acidity (TS/TA), Total sugars and Total phenols (TS/TP), glycerol content, ethyl acetate, amyl alcohols, 2-phenylethanol, minor esters (sum of 23 components) and minor alcohols (sum of hexanol, octanol, 4-vinylguaiacol and 4-vinylphenol). Four components explained more than 72% of the variance. In Figure 4 the projection onto the plane of the first two components is represented.

Table 3. Sensory profiles (citation frequencies, %) and overall quality (median of the tasting panel) of ciders according to apple juice type, yeast strain and harvest.

				Harvest	J1			J2			J3		
Attributes	J	S	H		C6	CHP	TD291	C6	CHP	TD291	C6	CHP	TD291
Candy	ns	ns	ns	1	28.6	14.3	28.6	0.0	0.0	0.0	57.1	42.9	14.3
				2	57.1	35.7	57.1	35.7	42.9	28.6	14.3	14.3	14.3
Fruity	**	ns	ns	1	14.3	28.6	35.7	41.7	50.0	33.3	57.1	71.4	71.4
				2	57.1	21.4	28.6	64.3	14.3	28.6	57.1	42.9	42.9
Apple	*	ns	ns	1	42.9	28.6	28.6	33.3	16.7	16.7	14.3	28.6	14.3
				2	57.1	28.6	57.1	42.9	28.6	0.0	28.6	28.6	28.6
Floral	ns	ns	ns	1	0.0	0.0	14.3	25.0	16.7	0.0	28.6	7.1	28.6
				2	14.3	14.3	14.3	14.3	0.0	0.0	14.3	28.6	14.3
Butter	***	ns	**	1	14.3	14.3	28.6	41.7	33.3	50.0	28.6	14.3	28.6
				2	0.0	0.0	0.0	21.4	28.6	28.6	14.3	14.3	21.4
Acidic/Fresh	ns	**	ns	1	14.3	0.0	28.6	33.3	16.7	16.7	14.3	21.4	57.1
				2	42.9	28.6	42.9	28.6	14.3	28.6	28.6	14.3	35.7
Bitter	ns	ns	*	1	57.1	42.9	14.3	25.0	33.3	16.7	57.1	28.6	28.6
				2	0.0	35.7	0.0	14.3	71.4	14.3	0.0	14.3	21.4
Astringent	ns	ns	**	1	14.3	28.6	14.3	41.7	33.3	33.3	28.6	21.4	28.6
				2	28.6	21.4	14.3	7.1	0.0	28.6	0.0	28.6	7.1
Overall Quality	ns	*	ns	1	3.0	3.0	3.0	2.0	2.0	2.5	2.0	2.0	2.5
				2	4.0	2.0	4.0	3.5	2.0	3.0	3.0	2.0	3.0

J: juice; S: strain; H: harvest. J1: *Durona de Tresali*; J2: *Perico + Limón Montés*; J3: *Verdialona + Regona + Durona de Tresali + Raxao + de la Riega*; ns: not significant; (*): significant at $p < 0.10$; (**): significant at $p < 0.05$; (***): significant at $p < 0.01$.
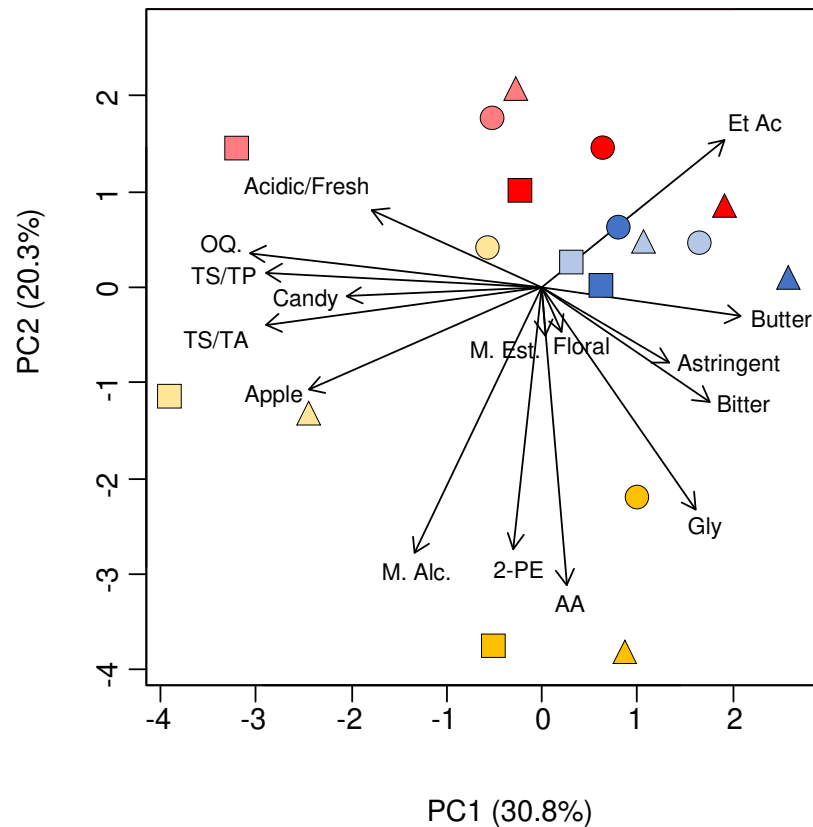
Figure 4. Projection of the ciders, chemical variables and sensory attributes onto the plane formed by the first two principal components. OQ.: Overall Quality; TS/TP: ratio of Total sugars and Total phenols; TS/TA: ratio of Total sugars and Total acidity; M. Est.: minor esters; M. Alc.: minor alcohols; 2-PE: 2-phenylethanol; AA: Amyl alcohols; Gly: Glycerol; EtAc: Ethyl acetate. Legends for samples: J1 □; J2 △; J3 ○. Yellow colour, *S. bayanus* C6; blue colour, *S. cerevisiae* Levuline-CHP; red colour, *T. delbrueckii* Biodiva-TD291. Lighter colours, ciders made during the second harvest.

The first axis is associated with ethyl acetate and attributes such as butter, astringent and bitter, with positive loadings; on this side are placed all the ciders made with the yeast strain Levuline-CHP, together with two samples obtained with the commercial non-*Saccharomyces* yeast strain (TD291). These ciders were made during the first harvest and with the apple juices J1 and J2. On the negative side of this axis the ratios TS/TA and TS/TP are projected together with sensory attributes such as candy, apple, acidic/fresh and the scores for overall quality. The ciders made from the single-cultivar apple juice (J1) and the yeast strains referred to as C6 and Biodiva-TD291 during the second harvest were closely linked to these variables. On the negative side of the second axis are placed five of the six ciders obtained with the autochthonous *S. bayanus* yeast strain, characterised by their higher contents of amyl alcohols, 2-phenylethanol and minor alcohols.

As reported elsewhere for ice ciders made by Cryo-extraction, the assessment of quality is positively related to the ratio TS/TA [9,14]. This association was also fulfilled in the ciders obtained by the Exhaustion method. The better scored ciders, those made from the single-cultivar apple juice fermented by the yeast strains *S.b.* C6 and *T.d.* TD291 (Table 3), presented the highest TS/TA values (Table 1). Attributes such as candy and apple were also associated with this ratio. This kind of interaction between basic tastes and aromatic stimuli has been previously observed in ice ciders [9].

The less valued ciders, obtained with the *S. cerevisiae* strain, are projected on the positive side of the first and second axes; in general, these ciders exhibited, in both harvests, the lowest TS/TA values, and the highest volatile acidities and alcoholic degrees (Table 1). The influence of ethanol on the perception of sweetness and bitterness has been investigated in different media. In model ice wine, Nurgel & Pickering [51] observed that, at lower

contents of sugars (around 150 g/L in their experiment), increasing levels of ethanol had no effect on sweetness perception; on the contrary, the bitterness perception increased as the level of ethanol was increased, depending on the sugar contents.

4. Conclusions

The valorisation method referred to as Exhaustion, associated with the Cryo-extraction method, allowed the creation of valuable ciders with different chemical and sensory profiles on the basis of the apple raw material and the yeast strain selected to conduct the fermentation. The three species of yeast tested were able to carry out this process successfully, although at different rates, providing ciders with contents of total sugars higher than 100 g/L, low volatile acidity and alcoholic degrees between 9 and 12 (% v/v), similar to those observed in ice ciders. The commercial *S. cerevisiae* was the most regular in terms of its ability to promote the alcoholic fermentation in the two harvests studied.

The freeze-enrichment system significantly influenced the aromatic profile of the ciders, with lower concentrations of 2-phenylethanol and all minor volatile compounds being observed in the ciders made by Exhaustion compared to the corresponding ice ciders made by Cryo-extraction. Even so, some of the ciders obtained by Exhaustion presented good quality scores, particularly those made during the second harvest with the *S. bayanus* and *T. delbrueckii* strains. For this reason, this process of valorisation of the must fractions discarded for the production of ice ciders by Cryo-extraction could be of interest to expand the offer of products derived from cider apple.

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