Ciders Produced by Two Types of Presses and Fermented in Stainless Steel and Wooden Vats

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ABSTRACT

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The aim of this study was to evaluate the influence of two factors – pressing (with either a traditional or a pneumatic press) and vat material (stainless steel and chestnut wood) – on the global parameters and volatile and acidic compounds of ciders throughout their fermentation in relation to micro-organism levels. A complete factorial design with two factors was applied and the samples were taken at five stages of cider fermentation. In each stage, 21 parameters were measured by standard methods, HPLC, GC, and enzymatic and microbiological techniques. Analysis of variance investigated the effect of pressing, vat types and replication of the measures on analytical and microbiological composition of the ciders. The results obtained for the different parameters indicated that the pneumatically pressed cider, fermented in a stainless steel vat, developed alcoholic and malo-lactic fermentations more slowly, and the decrease in phenolic compounds was also slower than it was for the traditionally pressed cider fermented in a wooden barrel. These differences influenced the composition of the cider.

Key words: Alcoholic fermentation, cider manufacture, malo-lactic fermentation.

INTRODUCTION

Cider production in the Basque Country (northern Spain) doubled between 1987 and 2002, becoming an increasingly important commercial product. The cider is generally produced by traditional methods: milling, pressing and re-pressing for about 24 h, followed by spontaneous clarification and natural fermentation of juice to dryness, mainly in chestnut barrels. This procedure has been used for several centuries; however, traditional presses and wooden barrels are now being replaced by new fermentation equipment, while maintaining traditional practices. In cider producing countries, most large producers are now using vertical stainless-steel tanks equipped with temperature control systems, and fermentation in wooden barrels is now only carried out by a few smaller manufacturers.

Four kinds of effects are attributed to barrels: evaporation, oxidation, extraction, and component reaction[14]. Evaporation through the staves is mainly influenced by container size, temperature, relative humidity and air circulation around the barrel[3]. Vacuum develops as ethanol and water evaporate from wine[12], and the effects of oxidation caused by oxygen are only evidenced in defective leaky barrels, or via the ullage space when it is opened and topped, or during racking. Extraction from wood compounds (mainly oak barrels) to the wine and subsequent reactions are responsible for the so-called woody character, producing a more complex flavor, considered a positive trait and not acquired in wines fermented in stainless steel vats. As repeated use of these barrels results in a significant reduction in extractable compounds, the barrels are frequently replaced. In cider production the barrels are generally reused for many years and the influence of extraction from the wood components to the cider is of little consequence. More important is the effect of the porous wooden structure on the retention of significant numbers of yeasts and bacteria, despite routine cleaning[15]. These microflora act as reservoirs of micro-organisms and affect the organoleptic profiles of ciders.

Cider quality depends on the transformations that the different apple juice components, mainly acids and sugars, undergo during the fermentation process. The nature and concentration of carboxylic acids are of interest in several aspects of cider making because they are used as a measure of taste, spoilage (acetic acid), and malo-lactic fermentation (L-malic and L-lactic acids).

As a consequence of alcoholic fermentation by yeasts, a number of volatile compounds are produced in the cider. Ethyl acetate is the most common ester present in ciders in amounts ranging from a few tenths to several hundred mg per liter[7,9,13]. The sensory importance of this ester lies in the synergistic action between the ester and the acetic acid, increasing the sour taste. Higher alcohols are also important components of the flavor profile; the levels formed depend on apple variety, juice treatment, yeast strain, fermentation and storage conditions[2]. Glycerol is, after water and ethanol, the main component of cider (1–4 g/L)[7,9,13] and also plays a significant role in sensorial quality; the conditions affecting the relative production of glycerol are

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yeast strain, temperature, pH, initial sugar concentration and aeration conditions[11].

The aim of this study was to evaluate the influence of two factors – pressing (with either a traditional or a pneumatic press) and vat material (stainless steel and chestnut wood) – on the global parameters and volatile and acidic compounds of ciders throughout their fermentation, in relation to micro-organism levels.

MATERIALS AND METHODS

Samples

The musts were obtained by mixing several varieties of Basque cider apples harvested in an experimental orchard in Hondarribia (Basque Country, northern Spain). The total production of the orchard, 6050 Kg of apples, was divided into two equal lots, washed, and milled with a grating mill. Half was pressed using the traditional pressing method (five squeezes over a 24 h period in a vertical mechanical press with a wooden platform). During pressing the must was continuously separated from pomace (without maceration) and kept refrigerated at a temperature of 9 ± 1°C. The juice obtained was subdivided (the abbreviations used in the text below are given in parentheses) in a 1000-L stainless steel tank (TS) and a 600-L traditional barrel made of chestnut wood (TW). All of the equipment was rigorously cleaned. The second lot of apples was pressed in a 1000 Kg capacity pneumatic press working in two cycles (first cycle at 0.6 bar and second cycle at 1.4 bar, total time: 280 min). This juice was also subdivided into a 1000-L stainless steel tank (PS) and a 600-L traditional barrel (PW). A dark room with temperature control at 15°C was conditioned to keep the vats during fermentation. In keeping with common practice, musts were not sulphited and the alcoholic and malo-lactic fermentations took place spontaneously by indigenous microflora. After alcoholic fermentation, a spontaneous keeving (known as défécation in French) was produced. Through the keeving process a gel rises to the top of the vat and a sediment deposits to the bottom, leaving a clear liquid layer in the middle. Ciders were then racked to remove these solids and to prevent autolysis of yeast with a release of micronutrients. Fermentation experiences were not replicated owing to limited apple supply and because the utilization of smaller vats was not considered representative of the process carried out by producers today.

Samples were taken at five stages of cider production: initial (stage 1, at 0 days), at the end of the alcoholic fermentation (stage 2, at 15 days), before and after racking (stages 3 and 4, at 35 and 48 days, respectively) and at the end of malo-lactic fermentation (stage 5, at 64 days). At each stage representative samples of the four tanks were taken and transported to the laboratory under refrigeration. There were a total of 20 cider samples. Samples from the stainless steel vats were collected by opening the spigot placed halfway up the tank; from the wooden vats the samples were aspirated from the centre of the barrel

Table I. Global parameter values in different stages of cider production using pneumatic (P) and traditional (T) presses, and stainless-steel (S) and chestnut wood (W) vats.

Fermentation stage	Parameter	PS	PW	TS	TW	Significance ANOVA
1 (Initial)	Turbidity (NTU)	1327A[1]	1327A	693B	693B	0.000
	Sec extract (g/L)	122.8A	122.8A	117.3B	117.3B	0.000
	Soluble solids (°Brix)	11.7A	11.7A	11.3B	11.3B	0.000
	pH	3.47A	3.47A	3.45A	3.45A	0.520
	Titratable acidity (meq/L)	66.7A	66.7A	68.6A	68.6A	0.133
	Phenolics (mg tannic acid/L)	879A	879A	820B	820B	0.000
2 (After 15 days)	Turbidity (NTU)	3368A	1985B	2047C	1081D	0.000
	Sec extract (g/L)	38.5A–B	39.3B	35.7A	40.2B	0.014
	Soluble solids (°Brix)	5.0A	5.2B	4.9A	5.3B	0.001
	pH	3.56A	3.57A	3.59A–B	3.61B	0.019
	Titratable acidity (meq/L)	57.0A	62.1B	66.1C	57.0A	0.000
	Phenolics (mg tannic acid/L)	846A	801A–B	776B–C	742C	0.001
3 (After 35 days)	Turbidity (NTU)	1141A	86B	410C	141D	0.000
	Sec extract (g/L)	21.6A	15.9B	18.0B	15.9B	0.002
	Soluble solids (°Brix)	3.5A	3.5A	3.5A	3.6A	0.863
	pH	3.59A	3.55B	3.69C	3.80D	0.000
	Titratable acidity (meq/L)	58.0A	65.3B	51.7C	45.3D	0.000
	Phenolics (mg tannic acid/L)	812A	710B	677B	703B	0.002
4 (After 48 days)	Turbidity (NTU)	967A	61B	83B	64B	0.000
	Sec extract (g/L)	21.4A	15.9B	15.4B	16.1B	0.000
	Soluble solids (°Brix)	4.1A	4.0A–B	3.9B	3.7C	0.001
	pH	3.63A	3.70B	3.84C	3.79D	0.000
	Titratable acidity (meq/L)	56.4A	54.1B	43.6C	53.1D	0.000
	Phenolics (mg tannic acid/L)	780A	690B	673B	662B	0.000
5 (After 64 days)	Turbidity (NTU)	634A	50B	110C	75D	0.000
	Sec extract (g/L)	18.8A	16.1B	12.9C	15.9B	0.000
	Soluble solids (°Brix)	4.0	3.7	3.5	3.5	0.000
	pH	3.65A	3.71B	3.74B	3.82C	0.000
	Titratable acidity (meq/L)	56.1A	52.1B–C	50.6C	55.2A–B	0.002
	Phenolics (mg tannic acid/L)	767A	691B	651B	659B	0.000

[1] Values in one row with an identical letter belong to an equal homogeneous subset at $p = 0.05$ according to the Tukey HSD multiple comparisons test.

through a hole on the top. Standard chemistry parameters (density, soluble solids, turbidity, pH, titratable acidity, and phenolic compounds) were measured in triplicate immediately after the samples were received at the laboratory. Samples for organic acid and volatile compound determination were centrifuged by using a temperature-controlled centrifuge (20000 g for 15 min at 5°C, Jouan MR 18.22, Saint Nazaire, France), filtered through a 0.45 µm Millex membrane (Millipore, Molsheim, France) and frozen at –20°C until analysis.

Standard chemistry

The parameters listed in Table I were analyzed as follows. Total soluble solids were measured as °Brix by a refractometer (Zuzi-300, QA Supplies LLC, Norfolk, USA). Turbidity was determined using a digital direct reading turbidimeter (model 2100P, Hach, Loveland, Colorado, USA) and reported in nephelometric turbidity units (NTU). Sec extract, pH, titratable acidity and Folin-Ciocalteu index were determined according to the MAPA procedures[10], and phenolic compounds content was calculated by reference to a calibration curve using tannic acid as standard. Sec extract is composed of the nonvolatile components (sugars, fixed acids, organic salts and other substances), and this is related primarily to the sugar content of the original must. The absorbance values were measured with a Shimadzu UV-260 spectrophotometer (Shimadzu, Kyoto, Japan).

Volatile compounds analysis

A gas chromatographic method was used for the analytical determination of the volatile compounds listed in Table II. Experimental data were obtained using a HP 6890 GC (Hewlett-Packard, Waldbronn, Germany) fitted with an HP Innowax (Hewlett-Packard, Waldbronn, Germany) (30 m × 0.25 mm × 0.25 µm) and equipped with an FID detector. Helium was used as carrier gas at 28 mL/min. The program temperature was: 3 min at 40°C, an increase to 65°C at 5°C/min, 1 min at 65°C, another increase to 85°C at 10°C/min, and 1 min at 85°C. Finally the oven temperature was increased to 170°C and maintained for 2 min to clean the column. The injector and detector temperatures were 200 and 250°C, respectively. The sample was filtered through a 0.22 µm Millex filter (Millipore, Molsheim, France) and 3 µL was directly injected (split mode) into the chromatograph. The compounds were identified by comparing the retention time with the peaks obtained from standard compounds. Quantitation was carried out by means of the internal standard method using 4-methyl-2-pentanol as internal standard.

Acids and glycerol analysis

Major organic acids listed in Table III were determined using a Spherisorb ODS-2 (Waters, Milford, MA, USA) (25 × 0.4 cm, 5 µm) and a Shimadzu 6A high performance liquid chromatograph (Shimadzu, Kyoto, Japan). The operating conditions were as follows: mobile phase 0.01 M

Table II. Volatile compounds content in different stages of cider production using pneumatic (P) and traditional (T) presses, and stainless-steel (S) and chestnut wood (W) vats.

Fermentation stage	Compound	PS	PW	TS	TW	Significance ANOVA
1 (Initial)	Methanol (mg/L)	41A[1]	41A	38A	38A	0.636
	n-Propanol (mg/L)	5.4A	5.4A	5.8A	5.8A	0.233
	n-Butanol (mg/L)	6.0A	6.0A	5.8A	5.8A	0.546
	Iso-butanol (mg/L)	0.0	0.0	0.0	0.0	—
	Iso-amyl alcohol (mg/L)	0.6A	0.6A	0.8B	0.8B	0.000
	Ethyl acetate (mg/L)	0.0	0.0	0.0	0.0	—
2 (After 15 days)	Methanol (mg/L)	43A–B	53B	44A–B	36A	0.018
	n-Propanol (mg/L)	8.4A	9.3B	8.7A–B	6.6C	0.000
	n-Butanol (mg/L)	11.2A	11.1A	25.8C	22.8D	0.000
	Iso-butanol (mg/L)	19.6A	28.2B	8.0B	8.2B	0.000
	Iso-amyl alcohol (mg/L)	11.3A	11.3A	12.6B	12.2B	0.000
	Ethyl acetate (mg/L)	4.1A	5.0B	8.0C	20.7D	0.000
3 (After 35 days)	Methanol (mg/L)	76A–C	66B	80C	69A–B	0.001
	n-Propanol (mg/L)	10.1A	10.1A	10.8A	11.9B	0.001
	n-Butanol (mg/L)	13.3A	13.1A	12.0B	12.2B	0.001
	Iso-butanol (mg/L)	26.3A	26.3A	34.5B	38.5C	0.000
	Iso-amyl alcohol (mg/L)	14.7A	14.6A	15.8B	18.3C	0.000
	Ethyl acetate (mg/L)	9.2A	19.2B	25.0C	30.1D	0.000
4 (After 48 days)	Methanol (mg/L)	83A	68B	90A	71B	0.001
	n-Propanol (mg/L)	9.5A	10.3B	12.2C	15.3D	0.000
	n-Butanol (mg/L)	12.2A	12.0A	12.1A	12.3A	0.546
	Iso-butanol (mg/L)	25.3A	31.3B	36.5C	46.8D	0.000
	Iso-amyl alcohol (mg/L)	14.4A	14.5A	16.1B	18.5C	0.000
	Ethyl acetate (mg/L)	11.6A	29.5B	37.0C	38.0D	0.000
5 (After 64 days)	Methanol (mg/L)	85A	67B	93A	71B	0.001
	n-Propanol (mg/L)	9.4A	9.4A	14.6B	16.9C	0.000
	n-Butanol (mg/L)	12.0A	12.1A	11.9A	12.2A	0.578
	Iso-butanol (mg/L)	24.8A	25.4A	36.5B	48.7C	0.000
	Iso-amyl alcohol (mg/L)	14.3A	14.3A	16.0B	18.8C	0.000
	Ethyl acetate (mg/L)	15.4A	31.3B	45.6C	48.7D	0.000

[1] Values in one row with an identical letter belong to an equal homogeneous subset at $p = 0.05$ according to the Tukey HSD multiple comparisons test.

KH_2PO_4/H_3PO_4 buffer, pH 2.7; flow rate 0.8 mL/min, column temperature, 20°C; ionic strength 0.01 M and injected volume 20 µL; column effluents were monitored at a wavelength of 210 nm. Acids were quantified by the external standard method from peak areas. Glycerol was enzymatically analysed by means of Roche reagents (kit E0148270, Roche Diagnostics, Mannheim, Germany).

Microbiological analysis

Yeasts, acetic acid and lactic acid bacteria were enumerated by spread-plating 0.1 mL aliquots of Ringer solution serial dilutions on malt agar (Difco, Detroit, USA), apple juice-yeast extract agar and MRS (Difco, Detroit, USA) supplemented with 20% tomato juice, respectively. For yeast growth, 100 mg/L of streptomycin and 105 IU/L of penicillin G were added to plating media to suppress the growth of acetic and lactic acid bacteria, respectively. In the case of acetic acid bacteria, a solution of 1% (v/v) of 0.5% pimaricin solution, and 1% (v/v) of penicillin G (2.5 × 105 IU) were added. To enumerate lactic acid bacteria, a solution of 1% (v/v) of 0.5% pimaricin solution and incubation was done in anaerobiosis (Gaspak, CO_2 + H_2, Biomerieux, France).

Statistical analysis

Statistical analysis of the results was carried out using the SPSS.11 statistical software package for Windows (SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) investigated the effect of press, vat types and analytical replication on the composition of the ciders throughout their fermentation, followed by a mean comparison test by Tukey HSD, which found homogeneous subsets for each data series.

RESULTS AND DISCUSSION

A comparison of the results in Tables I–III, for stage 1, shows that the most remarkable difference between the musts is the turbidity value. It is significantly higher ($p < 0.0001$) for the must obtained by pneumatic pressing (95% confidence interval: 1320–1338 NTU) than for that from traditional pressing (95% confidence interval: 686–701 NTU). This result is similar to that found by apple processing in a "rack and cloth" or "pack" press[4]. For the four musts the maximum turbidity value was measured in stage 2, mainly as a consequence of the high yeast populations (Table IV). The PS must showed significantly higher turbidity during fermentation with respect to the PW, TS and TW musts, and the influence of the vat material on the decrease in turbidity (comparison of PS and PW musts) was noticeable. After racking, the total sediments of each initial vat were examined, finding that the TS vat had more sediment than the PS vat, the TW more than PW vat, and the wooden barrels more than the stainless steel vats. Consequently, the cloudiest was the cider from the PS tank, and since the spontaneous keeving re-

Table III. Concentration of acids and glycerol in different stages of cider production using pneumatic (P) and traditional (T) presses, and stainless-steel (S) and chestnut wood (W) vats.

Fermentation stage	Compound	PS	PW	TS	TW	Significance ANOVA
1 (Initial)	Malic acid (g/L)	4.47A[1]	4.47A	4.56A	4.56A	0.666
	Lactic acid (g/L)	0.0	0.0	0.0	0.0	—
	Acetic acid (g/L)	0.0	0.0	0.0	0.0	—
	Pyruvic acid (mg/L)	0.0	0.0	0.0	0.0	—
	Succinic acid (g/L)	0.667A	0.667A	0.690A	0.690A	0.393
	Glycerol (g/L)	0.06A	0.06A	0.05A	0.05A	0.129
2 (After 15 days)	Malic acid (g/L)	3.90A	3.84A–B	3.62B	2.87C	0.000
	Lactic acid (g/L)	0.37A	0.35A	0.73B	1.14C	0.000
	Acetic acid (g/L)	0.0A	0.0A	0.26B	0.25B	0.000
	Pyruvic acid (mg/L)	112.0A	0.4B	51.3C	0.5B	0.000
	Succinic acid (g/L)	0.769A	0.777A	0.773A	0.783A	0.367
	Glycerol (g/L)	2.70A	2.94B	3.33C	3.03B	0.000
3 (After 35 days)	Malic acid (g/L)	3.16A	2.72B	1.82C	0.68D	0.000
	Lactic acid (g/L)	0.96A	1.08B	1.85C	2.47D	0.000
	Acetic acid (g/L)	0.02A	0.08B	0.36C	0.65D	0.000
	Pyruvic acid (mg/L)	110.0A	0.6B	47.4C	28.2D	0.000
	Succinic acid (g/L)	0.830A	0.822A	0.834A	0.869B	0.000
	Glycerol (g/L)	2.99A	3.38B	4.81C	4.83C	0.000
4 (After 48 days)	Malic acid (g/L)	1.97A	1.20B	0.75C	0.63D	0.000
	Lactic acid (g/L)	1.55A	2.12B	2.26C	2.42D	0.000
	Acetic acid (g/L)	0.02A	0.20B	0.43C	1.09D	0.000
	Pyruvic acid (mg/L)	76.4A	44.7B	23.6C	25.0C	0.000
	Succinic acid (g/L)	0.839A	0.844A	0.851A	0.925B	0.000
	Glycerol (g/L)	4.05A	4.27B	3.96C	3.94C	0.000
5 (After 64 days)	Malic acid (g/L)	1.18A	0.80B	0.63C	0.60C	0.000
	Lactic acid (g/L)	2.16A	2.37B	2.21A–C	2.28B–C	0.004
	Acetic acid (g/L)	0.02A	0.34B	0.75C	1.09D	0.000
	Pyruvic acid (mg/L)	39.3A	25.0B	19.2C	22.0B–C	0.000
	Succinic acid (g/L)	0.857A	0.892B	0.876B	0.989C	0.000
	Glycerol (g/L)	2.95A–C	3.13B	2.90A	3.07B–C	0.002

[1] Values in one row with an identical letter belong to an equal homogeneous subset at $p = 0.05$ according to the Tukey HSD multiple comparisons test.

duces the yeast and amino nitrogen content of the musts, this cider also had the highest yeast population (Table IV). In stage 1, there was also a significant effect of pressing technology ($p \leq 0.05$) on sec extract, soluble solids and phenolic compounds, whilst pH and titratable acidity showed significantly similar values. The pressing yield was similar: 0.62 and 0.64 L/Kg for pneumatic and traditional presses, respectively. Initially the must from the traditional press showed a significantly higher oxygen content (4.4 mg/L) than that from the pneumatic press (0.9 mg/L), but in stage 5 this content was very low for the four ciders due to anaerobic fermentation conditions. The sec extract values were highly correlated with those of soluble solids for the four ciders in the five stages (correlation coefficients higher than 0.996) and both values decreased during fermentation, although in stage 4 (after racking) a slight increase was observed for soluble solids. From stage 3 the extract value was significantly higher for PS cider, mainly as a consequence of the highest value for density, indicative of slower alcoholic fermentation.

The pH values increased throughout fermentation, and after 64 days (stage 5) the PS and TW ciders showed the lowest and the highest pH increase, respectively, whereas this value showed no significant differences between PW and TS ciders. Initially, the must from traditional pressing had lower phenolic content than that from the pneumatic press, because the longer stay time under aerobic conditions caused more rapid oxidation of these compounds[5]. Phenolic compounds content decreased during fermentation, more slowly for the PS cider, which from stage 3 maintained the highest content, whereas the PW, TS and TW ciders had similar significant phenolic content ($p \leq 0.05$). This result shows that in normal cider making conditions, the extraction of phenolic compounds from wood was negligible.

Alcoholic fermentation of the apple juice was monitored by the decrease in sec extract (dependent on density), the total soluble solids and by the production of alcohols and metabolic intermediates in glycolysis, such as pyruvic acid (Tables I–III), in relation to the yeast population (Table IV).

Methanol is not a direct product of fermentation; its major source is the pectinase activity which demethylates the methyl esters in pectins. There was no significant effect of pressing system on methanol content ($p = 0.636$), but from stage 3 it rose significantly for stainless steel vat ciders with respect to ciders from wooden barrels. This fact can be related with the presence of more suspended solids (higher turbidity) in the PS and TS ciders, which could contain methyl esters available for demethylation. For all ciders the methanol content found was lower than 100 mg/L. The initial musts contained amounts of n-propanol in the 5.4–5.8 mg/L range, and no significant effect was observed for pressing system ($p = 0.233$). However, beginning in stage 4 the traditionally pressed ciders showed a significantly higher n-propanol content than pneumatically pressed ciders, and in stage 5 equal homogeneous subsets were found for the four ciders for both n-propanol present in apple juice[1]. Initially and iso-amyl contents. n-Butanol is the main volatile compound the four musts had a similar content of this alcohol (5.8–6.0 mg/L) and the fermentation provoked a further increase up to double the initial content, regardless of the presses and vats used.

The production of higher alcohols is increased in the presence of finely divided pulp particles and by oxygenation[2]. Among these alcohols, iso-butanol and iso-amyl alcohols showed the highest increases after 15 days of fermentation. Throughout the fermentation, the content of iso-amyl alcohols was significantly higher in the musts from traditional pressing, probably related with the greater oxygenation of these musts. Moreover, from stage 3, each cider constituted a different subset; consequently, an interactive effect was found between press and vat for the TS and TW ciders. Iso-butanol and n-propanol production was higher for the PW cider in the stage 2, but in stage 5 the traditionally pressed musts reached the highest content in both alcohols, as well as in iso-amyl alcohols and in total higher alcohols. The effect of the initial aeration of the musts during traditional pressing probably had more influence on the production of higher alcohols than the high levels of turbidity found for the musts from pneumatic pressing.

Table IV. Development of yeasts, acetic and lactic acid bacteria in different stages of cider production using pneumatic (P) and traditional (T) presses, and stainless-steel (S) and chestnut wood (W) vats.

Fermentation stage	Microorganisms (log CFU/mL)	PS	PW	TS	TW	Significance ANOVA
1 (Initial)	Yeasts	6.70A[1]	6.70A	6.04B	6.04B	0.000
	Acetic acid bacteria	5.77A	5.77A	5.26B	5.26B	0.002
	Lactic acid bacteria	4.08A	4.08A	4.37B	4.37B	0.008
2 (After 15 days)	Yeasts	7.28A	7.30A	7.23A	7.35A	0.421
	Acetic acid bacteria	4.40A	4.12B	3.21B	4.30B	0.002
	Lactic acid bacteria	4.68A	4.73A	5.47B	6.26C	0.000
3 (After 35 days)	Yeasts	6.97A	5.71A	6.19A	5.62B	0.003
	Acetic acid bacteria	2.62A	2.69A	2.36A	2.42A	0.253
	Lactic acid bacteria	5.16A	5.57B	6.50C	7.85D	0.000
4 (After 48 days)	Yeasts	6.19A	4.76A	4.00B	4.07C	0.000
	Acetic acid bacteria	1.67A	1.77A–B	1.56A–B	1.11B	0.035
	Lactic acid bacteria	6.15A	7.21B	7.31C	8.04C	0.000
5 (After 64 days)	Yeasts	5.49A	3.58B	2.63B	3.60C	0.001
	Acetic acid bacteria	1.00A	1.18B	1.30B	1.66B	0.059
	Lactic acid bacteria	7.00A	7.58A	7.45B	7.09B	0.006

[1] Values in one row with an identical letter belong to an equal homogeneous subset at $p = 0.05$ according to the Tukey HSD multiple comparisons test.

From an acetic acid concentration higher than 0.08 g/L, the ethyl acetate content was well correlated with that of acetic acid ($r > 0.95$) (Tables II–III). There was a significant effect of the pressing technology on ethyl acetate content. Moreover, the four ciders formed four subsets throughout fermentation, indicating that an interactive effect between the press and vat factors was produced. Thus, in stage 5, PS, PW, TS and TW ciders contained 15.4, 31.3, 45.6 and 48.7 mg/L of this ester, respectively.

Pyruvic acid was not found in the initial musts. It is a metabolic intermediate in glycolysis, which is excreted by yeasts during fermentation. As can be seen in Tables III–IV, in stages 2–5 the pyruvic acid content was significantly higher in the PS cider than in the PW, TS and TW ciders. A correlation analysis showed that for PS cider the log pyruvic content was highly correlated with log yeast population ($r = 0.966$; stages: 2, 3, 4 and 5), and log yeast population with log turbidity ($r = 0.962$; stages: 3, 4 and 5). It is likely that high turbidity favours yeast survival and, consequently, pyruvic acid production. In stage 4, the following three homogeneous subsets ($p = 0.05$) were found for pyruvic acid content: (PS), (PW) and (TS-TW). They corresponded with the subsets for glycerol content and yeast population (Tables III–IV), showing a significant effect of pressing system and also an interactive effect between press and vat types for the ciders from pneumatic pressing.

Glycerol is a product of alcoholic fermentation and its yield is generally related to that of succinate. For the four ciders studied, glycerol and succinic acid yields were well correlated over the earlier stages of fermentation: stages 1–4 for PS and PW ciders, and stages 1–3 for TS and TW ciders, with correlation coefficients from 0.965 to 0.992. Whilst glycerol is only produced by alcoholic fermentation, succinic acid is produced by two ways: (a) through a pathway of yeast metabolism, from pyruvate, and (b) from malate through malo-lactic fermentation under anaerobic conditions. In (b) the ratio between lactic and succinic acids produced varies with the pH, as was found for *L. collinoides,* which produced more lactic than succinic acid at pH 3.6, but succinic acid only at pH 4.8[2]. We observed that the succinic acid production continued after completed alcoholic fermentation, with decreasing yeast populations, but with increasing lactic acid bacteria populations (Table IV). Thus, the succinic acid content should be the result of the succinic acid from the must, together with that produced by yeasts during alcoholic fermentation, and also of succinic acid produced by some lactic acid bacteria during malo-lactic fermentation. In stages 4 and 5 these contents were significantly higher for TW cider, in accordance with its high pH values and lactic acid bacteria populations (Tables III–IV). In respect to glycerol, the main difference between the four ciders was the higher production rate for the traditionally pressed musts, which reached their highest glycerol content after 35 days. In the PS and PW ciders, however, the content increased until stage 4 (48 days) of fermentation. Finally, in stage 5, all ciders showed a decrease in glycerol concentration, but maintained significantly similar contents. Glycerol can be degraded by lactic acid bacteria in two different ways. It can be a substrate for glycerol dehydrogenase, producing lactic, acetic or other organic acids depending on the bacteria, or for glycerol dehydratase with 1,3-propanediol production[6] thus decreasing the glycerol content.

Malo-lactic fermentation (MLF) was monitored by the decrease of malic acid and increase of lactic acid (Table III) in relation to the populations of lactic acid bacteria (Table IV). The variation of titratable acidity (Table I) during fermentation was also dependent on the L-malic to L-lactic conversion. The initial must from traditional pressing, showed lactic acid bacteria populations significantly higher than those from pneumatic pressing, although in this stage no significant differences were found according to their malic, lactic or acetic acids contents. In stage 3 and 4 the statistical analysis found significant differences between the four ciders, which were classified into four homogeneous subsets with respect to lactic acid bacteria populations, malic, lactic and acetic acid content, and also according to titratable acidity. These results showed that the rate of the MLF was influenced by the type of press and vats, decreasing as follow: TW > TS > PW > PS ciders.

Previous work showed that during the maturation of cider, extensive growth of lactic acid bacteria can occur, especially if wooden vats are used[8]. In our experience this effect was verified and the results showed an interaction between the types of press and vat used. Thus, the use of a wooden press had a significant effect on the increase of lactic acid bacteria populations, which were further increased with wooden barrels. Lactic acid bacteria must be responsible for the high levels of acetic acid content. Acetic acid bacteria can also be acetic acid producers, but as seen in Table IV, this population decreased quickly in the four vats during fermentation and, consequently, their influence on acetic acid production would be negligible. On the other hand, the musts from the stainless steel press had lower lactic acid bacteria populations, which grew more slowly for the ciders fermented in stainless steel tanks; consequently the PS cider had low acetic acid content.

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REFERENCES

1. Acree, T.E. and McLellan, M.R., Flavor components and quality attributes. In: Processed Apple Products, D.L. Downing, Ed., Van Nostrand Reinhold: New York, 1989, pp. 323–339.
2. Beech, F. W., Cider making and cider research: a review. *J. Inst. Brew.,* 1972, **78**, 477–491.
3. Blazer, R.M., Wine evaporation from barrels. *Proc. Wine Vineyard,* 1991, **12**, 20–23.
4. Bump, V.L., Apple pressing and juice extraction. In: Processed Apple Products, D.L. Downing, Ed., Van Nostrand Reinhold: New York, 1989, pp. 53–80.
5. Cheynier, V., Masson, G. and Moutounet, M., Estimation of must oxidation during pressing in Champagne. *Am. J. Enol. Vitic.,* 1993, **44**, 393–399.
6. Claisse, O. and Lonvaud-Funel, A., Assimilation of glycerol by a strain of *Lactobacillus collinoides* isolated from cider. *Food Microbiology,* 2000, **17**, 513–519.

7. Irastorza, A., Munduate, A., González, A., Aierbe, T. and Del Campo, G., Caractéristiques analytiques des cidres de la province de Guipuzcoa (Pays Basque*). J. Int. Sci. Vig. Vin,* 1993, **27**, 55–63.
8. Jarvis, B., Forster, M.J. and Kinsella, M.J., Factors affecting the development of cider flavour. *J. Appl. Bact. Symp. Suppl.,* 1995, **79**, 5S–18S.
9. Leguerinel, I., Cleret, J.J., Bourgeois, C.M. and Mafart, P., Essai d'evaluation des caractéristiques organoleptiques des cidres par analyses instrumentales. *Sci. Aliments,* 1987, **7**, 223–228.
10. Ministerio de Agricultura, Pesca y Alimentación, MAPA, Sidras. In: Métodos Oficiales de Análisis. Tomo II, Secretaría General Técnica: Madrid, 1993, pp. 359–365.
11. Ough, C.S. and Amerine, M.A., 1988. Alcohols. In: Methods for Analysis of Musts and Wines, 2nd ed., C.S. Ough, Ed., Wiley: New York, pp. 80–139.
12. Peterson, R.G., Formation of reduced pressure in barrels during wine aging. *Am. J. Enol. Vitic.,* 1996, **27**, 80–81.
13. Picinelli, A., Suárez, B., Moreno, J., Rodríguez, R., Caso, L.M., Pando, R.M. and Mangas, J., Técnicas analíticas en el control de calidad y caracterización de la sidra natural asturiana. *Alimentaria,* 2000, **9**, 129–136.
14. Singleton, V.L., Maturation of wines and spirits: Comparisons, facts and hypotheses. *Am. J. Enol. Vitic.,* 1995, **46**, 98–115.
15. Swaffield, C.H. and Scott, J.A., Existence and development of natural microbial populations in wooden storage vats used for alcoholic cider maduration. *J. Am. Soc. Brew. Chem.,* 1995, **53**, 117–120.

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