Influence of cider-making technology on cider taste

Juan Mangas<sup>1</sup>, Carmen Cabranes<sup>1</sup>, Javier Moreno<sup>1</sup> and Domingo Blanco<sup>2</sup>

- 1 Instituto de Experimentación y Promoción Agraria, E-333000 Villaviciosa, Asturias, Spain
- 2 Departamento de Quimica Física y Analítica, Universidad de Oviedo, E-33006 Oviedo, Spain

Einflueta der Herstellungsmethoden auf das Geschmack des erhaltenen Apfelweines

Zusammenfassung. Die Hauptgeschmackskomponenten des Apfelweines (Organischensäuren, Kohlenhydraten, Alkoholen und Pektinen) sowie die verantwortlichen Mikroorganismus der Gärung aus zwölf Apfelweinen, die mit verschiedenen technischen Methoden erhalten wurden, wurden analysiert um die kualität des erhaltenen Apfelweines zu bestimmen. Die erhaltenen Daten wurden durch Varianze Analysen in Funktion der  $Pre\beta$ geschwindigkeit und des Klärungssystems als Hauptvariablen bearbeitet.

Abstract. Some major cider taste components (organic acids, sugars, alcohols and pectin substances) together with the microorganisms responsible for fermentations were determined in twelve ciders obtained by different technological methods in order to evaluate the quality of cider obtained. The data obtained was submitted to variance

analysis taking speed of pressing and the clarification system as the main factors.

## Introduction

The production of traditional cider is carried out by sequential apple juice extraction using hammer mills and mechanical presses, followed by natural fermentation using wild microflora of the fruit or that residing on the press and fermentation casks. These traditional methods as well as being very slow and expensive can cause uncontrolled fermentations that determine strong variations in the cider quality, especially if the raw material lacks of sanitary conditions and the temperature is too high during the juice Moreover, the extraction. spontaneous apple juice clarification, which depends on calcium, pectin, malic acid and polyphenol contents in addition to pH and pectin methyl esterase activity, causes unstable ciders.

Now, for apple juice extraction, three methods are employed: pressing, hot diffusion and liquefaction. Initially, for pressing, rack and frame presses and later semi-automatic horizontal basket presses were used[1,2]. Actually, automated pneumatic presses with working pressures up to 90 psi were preferred. Belt and screw presses[3] are another press type used in juice-making technology but, in order to keep the mash compact and the juice conduit free, press aids are neccesary.

The modern hot diffusion technique, considered as an osmosis through vegetal cell[3], and liquefaction, consisting of an enzymatic digestion of vegetal cell with a releasing of vacuolar and citoplasmatic juices[4], used to make juice concentrates could be used in cidermaking technology even though the experiences carried out still don't allow the assertion that these systems are a better alternative to modern presses.

Between the different methods used for apple juice clarification (enzyme clarification and fining, enzymatic keeving), we believe that enzymatic keeving is the best choice. If the enzymatic activity is enough and if in the apple juice there exists sufficient amounts of calcium and pectin substances, a calcium pectate gel is shaped trapping a fraction of the microorganisms. As a consequence, the fermentations are carried out slower and more aromatic and stable ciders are obtained[5].

The aim of this paper is to evaluate the effect on the main cider taste components of new technological methods employed in its processing as an alternative to traditional methods.

#### Material and methods

Cider. The cider used during this work was prepared from the juice of a mixture of cider apples with different sensory properties giving the resulting juice an overall acidic nature. Such a mixture was processed in a pilot plant using two technological procedures: traditional and alternative

Traditional cider-making technology employs systems. unwashed apples and involves milling with a hammer mill and slow pressing with a batch mechanical press (4 day pressing time). The alternative system employs washed apples and involves milling with a grating mill and a fast press cycle with a rack and frame press (30-min pressing time plus 15 hr of maceration). From the must obtained from both systems we investigated the effects of spontaneous and enzymatic clarification. This last system was carried out by adding 1,200 units/Hl of pectin methyl esterase (rapidase CPE-Gist-Brocades) and 10 mmol of Ca2+ as dihydrate calcium chloride to the apple juice. Alcoholic fermentation and malolactic transformation were allowed to take place by natural microflora , mainly Saccharomyces cerevisiae and Leuconostoc oenos.

Liquid chromatography. A liquid chromatograph equipped with a Waters 510 pump, a Wisp 712 automatic injector, a Waters 410 RI detector and a Digital 380 data station was used to determine carbohydrates and alcohols. The Sugar Pak I column used (300x6.5 mm i.d.) from Waters Associates was operated under the following conditions: mobile phase, water containing 50 ppm Ca(Na)<sub>2</sub>EDTA; flow-rate, 0.5 mL/min; column temperature, 80°C; detector temperature, 37°C; detector sensitivity, 32; injected volume, 10 μL [6].

Organic acids were determined with the same chromatograph equipped with a Waters 990 photodiode array detector using a Spherisorb ODS-2 column (3  $\mu$ m, 250 x 4 mm i.d.). Column effluents were monitored at a wavelength of

206 nm. The operating conditions were as follow: mobile phase 0.01M  $KH_2PO_4/H_3PO_4$  buffer, pH=2.25; flow-rate, 0.5 mL/min; column temperature, 25°C; injected volume, 20  $\mu$ L [7].

Other analytical methods. D(-) lactic acid was analysed by the enzymatic procedure developed by Boehringer and Mannhein [8]. For the AAS measurements of calcium, a Perkin-Elmer Model 3030 Atomic Absorption Spectrometry was used. calcium hollow cathode lamp was set at 422.7 nm conditions according to manufacture's instructions. pectin was measured as total galacturonic acid incubation with sulfuric acid and subsequent color development with alkaline m-hydroxydiphenyl [9,10] after alcohol pectin precipitation. The process was monitored spectrophotometrically at 520 nm on a Perkin-Elmer Landa 5 instrument.

#### Results and discussion

We monitored the changes in each of the major sugars (sucrose, glucose and fructose), alcohols (glycerol and ethanol), organic acids (L(-)malic, L(+) and D(-) lactic, succinic, shiquimic and acetic), pectin and calcium contents, together with the microorganisms responsible for fermentations, in twelve ciders obtained according to the methods shown in Fig.1.

Analysis of variance [11] was performed on data obtained with speed of pressing and the clarification system as the main factors.

In the first stages of the process, there was a significant effect of speed of pressing on the apple juice pectin contents (P=0.0001). The higher content of pectin obtained using the traditional pressing system (960.5  $\mu$ g/mL) respective to the alternative system (769.3  $\mu$ g/mL) can be explained as a consequence of the structure and location of pectin substances in the vegetable cells. The high molecular weight of these compounds result in slow diffusion rate from the middle llamella and wall cell to juice. On the other hand, the extracted clarification steep by enzymatic keeving, the contents decrease (57.8% and 74.9% for traditional and alternative systems, respectively; P=0.0001) consequence of trapping, together with the microorganisms, nitrogen substances and impurities, by the calcium pectate gel formed. On the contrary, the calcium amounts during enzymatic keeving increase. If we compare the initial calcium concentration in the juice (35.83 ppm), the calcium added (400 ppm) and the calcium present in the juices after enzymatic keeving (345 ppm), it can be inferred that the greater part of the calcium remains in the liquid phase complexed by malic acid.

In the same way, the initial concentrations of sucrose, ethanol, malic and acetic acids, yeasts and acetic bacterias (Tables 1,2 and 3) were conditioned by the pressing system employed: P= 0.0001, 0.001, 0.0001, 0.0001

and 0.009, respectively. With the traditional system, sucrose is more easy hydrolyzed, more malic acid is conversed and more ethanol and acetic acid are generated. These results are in accordance with the larger population of yeast and acetic bacteria obtained with this technique as a consequence of the larger pressing time under aerobic conditions.

In the first stages of alcoholic fermentation, tumultuous fermentation, the malic acid decreases notably in all cases, probably due to great zymological activity (Table 3). It is noticeable in this stage that the amounts of L(+) lactic acid produced are not equivalent to the malic acid transformed. On the contrary, succinic acid concentration increases quickly due to sugars, malic acid and aminoacids metabolising. It is also noticeable in this stage that the traditional pressing system, irrespective of the clarification system employed, provided more acetic acid (Table 2).

During slow fermentation, the malolactic conversion took place in all experimental units except in those obtained by the fast pressing and enzymatic keeving system (Table 2). This is in accordance with the lactic bacteria cellular density measured (Table 3). Simultaneous to malolactic conversion, the succinic acid concentration increase was probably due to malosuccinic fermentation. In a few cases, the lactic acid generated is too high compared with the malic acid consumed. This trend can be explained by the lactic bacteria glycolysis in order to obtain enough energy for its exponential growth. It is possible to

observe in this stage, employing the slow pressing system, that the amounts of D(-) lactic and acetic acids (Table 2) increase simultaneously, probably as a consequence of major availability of carbonaceous sources (Table 1).

During cider maturation, there was a significant effect of the main factors studied on fructose, L(+) and D(-) lactic, acetic and shiquimic acids and lactic bateria contents (Tables 1,2 and 3). Partial malolactic conversion was experienced by the ciders made by the fast pressing and enzymatic keeving system. Again, a correlation between D(-) lactic acid, acetic acid and residual sugars contents were detected with the traditional pressing system, and shiquimic acid decreased as a consequence of anaerobic oxidation process.

After the cider was bottled, there was a significant effect of the speed of pressing on fructose, glycerol, ethanol and L(+) lactic, D(-) lactic and shiquimic acids. Thus, a higher glycerol and lower ethanol content was obtained by the slow pressing system indicating that the activity over alcoholic and glycero-pyruvic fermentations was conditioned by the technology employed. With respect to organic acids, shiquimic and L(+) lactic acid decreased probably as a consequence of anaerobic oxidation processes with acetic acid formation. Also, it is possible that the L(+) lactic was transformed into acetoinics substances. On the contrary, D(-) lactic acid increased simultaneously to a glycerol decrease, indicating some interaction.

Also, a numerical scoring test was carried out for the ciders obtained. Several factors were scored, namely: colour, taste, flavour, turbidity, greasiness, foam stability and carbonation. The statistical analysis of the data was carried out according to the analysis of variance. The null hypothesis is not rejected at the 5% significance level, since Fpressing, Fclarification and Finteraction are smaller that F0.05:1.8 = 5.32, therefore no sensorial differences were found in the ciders for the factors studied: pressing, clarification and interaction.

We can conclude that the slow pressing with enzymatic keeving makes ciders with more fermentable sugars, specially fructose, therefore more microbiological disorders can take place. Employing the fast pressing and enzymatic keeving system, alcoholic fermentation and malolactic conversion take place separately and the cider obtained is better microbiologically stabilized, without sensorial change with regard to traditional methods. From an economical and technological point of view, the alternative system is more recommendable.

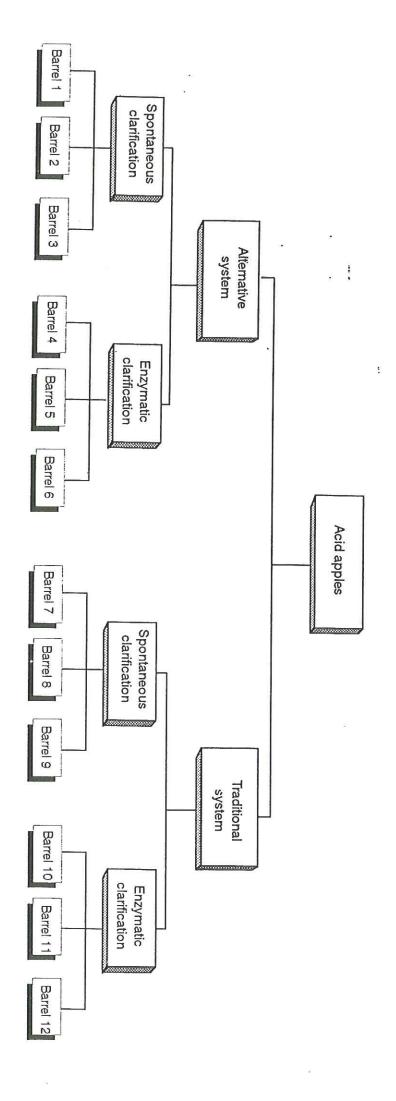
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# FIGURE CAPTIONS

Fig.1. Experimental design of the pilot plant



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Table 1. Sugar and alcohol contents (g/L) in different stages of cider production. Pp = pressing confidence level, Pc = clarification confidence level.

Fermentation stages	Technologies Pressing-clarification	Sucrose	Glucose	Fructose	Glycerol	Ethanol
Initial	Fast - Spontaneous	30.02	21.26	68.24	0.83	0.37
	Fast - Enzymatic	30.02	21.26	68.24	0.83	0.37
	Confidence level	Pp = 0.0001	Pp>0.1	Pp>0.1	Pp>0.1	Pp = 0.000
	Slow - Spontaneous	25.89	22.25	66.71	0.37	4.00
	Slow - Enzymatic	25.89	22.25	66.71	0.37	4.00
10	Fast - Spontaneous	5.35	10.78	45.65	2.37	31.22
*	Fast - Enzymatic	2.69	14.24	47.85	3.18	32.05
Tumultuous	Confidence level	Pp = 0.01 Pc = 0.0019	Pp=0.0124	Pc=0.0224	Pp,c>0.1	Pp,c>0.1
	Slow - Spontaneous	8.18	13.70	37.00	2.94	30.13
	Slow - Enzymatic	20.01	13.25	41.40	2.66	26.18
Slow	Fast - Spontaneous	0.48	0.63	9.86	3.65	51.35
	Fast - Enzymatic	0.07	0.19	4.23	4.13	60.11
	Confidence level	Pp,c>0.1	Pp=0.0001 Pc=0.0012	Pc=0.0255	Pp,c>0.1	Pp,c>0.1
	Slow - Spontaneous	0.20	2.08	14.29	3.72	53.41
	Slow - Enzymatic	5.74	3.64	12.89	4.05	48.74
	Fast - Spontaneous	0.44	0.45	1.79	5.54	61.06
Maturation	Fast - Enzymatic	0.28	0.43	0.37	4.45	60.12
and storage	Confidence level	Pp,c>0.1	Pp,c>0.1	Pc=0.0247	Pp,c>0.1	Pp,c>0.1
	Slow - Spontaneous	0.15	0.39	0.15	5.20	55.92
	Slow - Enzymatic	0.45	0.94	4.49	6.15	58.37
Bottled	Fast - Spontaneous	0.47	0.42	1.57	3.97	57.87
	Fast - Enzymatic	0.00	0.55	0.08	2.97	59.54
	Confidence level	Pp,c>0.1	Pp,c>0.1	Pp=0.063	Pp = 0.0002	Pp=0.0151
	Slow - Spontaneous	0.46	0.12	0.89	4.64	57.25
	Slow - Enzymatic	0.02	0.54	5.01	5.37	54.40

Table 2. Organic acid contents (g/L) in different stages of cider production. Pp = pressing confidence level, Pc = clarification confidence level.

Fermentation stages	Technologies Pressing-clarification	Malic	L(+) Lactic	D(-) Lactic	Succinic	Acetic	Shiquimic
Initial	Fast - Spontaneous	5.81	0.01	0.00	0.02	0.00	0.02
	Fast - Enzymatic	5.81	0.01	0.00	0.02	0.00	0.02
	Confidence level	Pp = 0.0001	Pp>0.1	Pp>0.1	Pp>0.1	Pp=0.0001	
	Slow - Spontaneous	4.42	0.00	0.00	0.02	0.09	0.02
	Slow - Enzymatic	4.42 ·	0.00	0.00	0.02	0.09	0.02
Tumultuous	Fast - Spontaneous	4.36	0.48	0.07	0.63	0.01	0.02
	Fast - Enzymatic	4.14	0.27	0.06	0.58	0.10	0.02
	Confidence level	Pp,c>0.1	Pp = 0.0268 Pc = 0.0020	Pρ,c>0.1	Pp,c>0.1	Pp=0.0018	••••••••
	Slow - Spontaneous	3.17	0.09	0.06	0.54	0.42	0.02
	Slow - Enzymatic	3.40	0.00	0.04	0.59	0.41	0.02
Slow	Fast - Spontaneous	0.45	4.09	0.15	0.91	0.10	0.03
	Fast - Enzymatic	4.58	0.49	0.07	0.75	0.18	0.02
	Confidence level	Pp = 0.0058 Pc = 0.0142	Pc = 0.0445	Pp=0.0113	Pp=0.0049	Pp = 0.0351	Pp,c>0.1
	Slow - Spontaneous	0.00	2.44	0.35	0.74	0.57	0.02
	Slow - Enzymatic	0.16	2.48	0.39	0.76	0.73	0.02
	Fast - Spontaneous	0.00	4.61	0.32	0.75	0.44	0.03
	Fast - Enzymatic	1.58	2.23	0.17	0.79	0.31	0.02
Maturation and storage	Confidence level	Рр,с>0.1	Pp = 0.0001 Pc = 0.0331	Pp=0.0039	Pp,c>0.1	Pp=0.0001	
	Slow - Spontaneous	0.02	2.12	1.42	0.61	1.35	0.01
	Slow - Enzymatic	0.00	2.26	1.14	0.48	1.23	0.02
Bottled	Fast - Spontaneous	0.00	2.21	2.67	0.66	0.78	0.00
	Fast - Enzymatic	1.76	1.68	1.91	0.48	1.00	0.00
	Confidence level	Pp,c>0.1	Pp=0.0079 Pc=0.004	Pp = 0.0002	Pp,c>0.1	Pp,c>0.1	Pp = 0.0001
	Slow - Spontaneous	0.00	1.43	1.42	0.48	1.44	0.00
	Slow - Enzymatic	0.00	2.08	1.82	0.53	1.14	0.01

Table 3. Yeast and Bacteria populations (Log cfu/mL) in different stages of cider production. Pp = pressing confidence level, Pc = clarification confidence level.

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Fermentation stages	Technologies Pressing-clarification	Yeast	Lactic Bacteria	Acetic Bacteria
	Fast - Spontaneous	6.06	4.41	4.95
	Fast - Enzymatic	6.06	4.41	4.95
Initial	Confidence level	Pp=0.0001	Pp>0.1	Pp=0.0088
	Slow - Spontaneous	7.29	4.34	5.12
	Slow - Enzymatic	7.29	4.34	5.12
	Fast - Spontaneous	7.39	4.53	4.48
6	Fast - Enzymatic	7.31	4.73	2.74
Tumultuous	Confidence level	Pp=0.0233	Pp.c>0.1	Pp,c>0.1
	Slow - Spontaneous	6.40	4.67	4.19
	Slow - Enzymatic	6.54	4.66	4.22
	Fast - Spontaneous	5.30	6.27	2.29
	Fast - Enzymatic	4.98	5.05	1.25
Slow	Confidence level	Pp,c>0.1	Pp,c>0.1	Pp,c>0.1
	Slow - Spontaneous	4.04	6.36	2.11
	Slow - Enzymatic	4.69	6.43	2.07
	Fast - Spontaneous	4.22	5.28	1.84
Maturation	Fast - Enzymatic	4.19	5.18	1.01
and storage	Confidence level	Pp,c>0.1	Pc = 0.0001	Pp,c>0.1
	Slow - Spontaneous	3.76	5.40	2.13
	Slow - Enzymatic	4.14	5.88	1.59
	Fast - Spontaneous	2.87	3.59	1.50
( <b>*</b>	Fast - Enzymatic	2.73	4.32	0.74
Bottled	Confidence level	Pp,c>0.1	Pp,c>0.1	Pp,c>0.1
	Slow - Spontaneous	1.84	2.96	0.77
	Slow - Enzymatic	3.11	4.63	0.52