Aromatic Profile of Ciders by Chemical Quantitative, Gas Chromatography-Olfactometry, and Sensory Analysis

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Abstract: Nine samples of Asturias cider have been analyzed for volatile, olfactometric, and sensorial profiles. The aromatic composition was mainly constituted by fusel alcohols and ethyl esters. Among the minor volatile compounds, fatty acids, volatile phenols, and alcohols were the main components. The olfactometric analysis revealed the existence of 55 aromatic areas, exhibiting a wide range of intensities. Components like amyl alcohols, 2-phenylethanol, ethyl esters such as 2-methylbutyrate, hexanoate and octanoate, hexanoic and octanoic acids 2-phenylethyl acetate, 4-ethyl guaiacol, and 4-ethyl phenol could be considered as being part of the structure of cider aroma. The extract dilution analysis of one extract identified 2 volatile phenols (4-ethyl guaiacol and 4-ethyl phenol) among the most powerful odorants in cider. These components gave significant correlations with the sensory attributes sweet, spicy, and lees.

Keywords: aromas, chemical composition, cider, GC-olfactometry, sensory evaluation

Practical Application: Although cider is a popular and worldwide beverage, only a few and partial studies have been focused to the knowledge of its aromatic composition. However, the characteristic aroma profile of cider suggests that some concepts relating high contents of volatile phenols or acetic acid with sensory defects should be revised in the case of this beverage.

Introduction

Odor and flavor are the most important quality factors in an alcoholic beverage, and they are closely related to the making procedure. Therefore, the study of the aroma of foods and beverages is a challenging but difficult task, involving gas chromatographic, olfactometry, and sensory techniques.

Cider-making is one of the most relevant agro-food industries in Asturias, a region located on the Atlantic coast of northern Spain. Production rates of cider (sparkling and still cider) place this region in the 4th position of Europe, following the United Kingdom, Ireland, and France (http://www.aicv.org). This product is recognized as Protected Designation of Origin by the European Union (Commission Regulation Nr. 2154/2005), and takes into account 2 products: still and sparkling cider. The making of cider follows a traditional method which includes pressing of cider apples and spontaneous alcoholic fermentation and malolactic transformation by the autochthonous microorganisms typically associated to each cellar year after year (Suárez-Valles and others 2007a), giving rise to characteristic cellar profiles. Although cider is a popular and well-known beverage in Europe, to the best of our knowledge there are only a few quantitative studies about its aroma profile. The volatile composition of cider is characterized by the presence of fusel alcohols, ethyl acetate, and ethyl lactate as major compounds (Picinelli and others 2000), together with short- and medium-chain fatty acids, esters, and carbonyls (Mangas and others 1996; Wang and others 2004; Rodríguez Madrera and others 2005; Xu and others 2007; Rodríguez Madrera and others 2008; Satora and others 2008, 2009). Moreover, olfactometric researches on cider aroma are even scarcer. For instance, Xu and others (2007) described the olfactometric profiles of 2 single-cultivar apple ciders by measuring olfactory intensities, comparing the results obtained by using different methods for the extraction of the volatile compounds and Villière and others (2012) compared 2 headspace methods to identify odor active compounds in sweet commercial French ciders.

The aims of this paper were to quantify the aromatic composition of natural cider to seek for odor-active and potentially discriminatory compounds in cider on the basis of their olfactometric and sensory profiles. To measure the olfactometric data, a technique combining detectionfrequency and odor intensity of the eluting components was chosen.

Materials and Methods

Cider samples

Nine samples of cider (6 bottles each) made in the 2008 harvest were taken at bottling at their respective cellars and kept at 12 °C until chemical and sensory analysis, for a maximum of 1 mo. These ciders were made with different apple mixtures, according to the particular conditions used by the respective cider makers, under the rules of the Protected Designation of Origin regulation. They were obtained by spontaneous fermentations excepting those referred to as C4, C6, and C10, which were made by using commercial *Saccharomyces* strains. The chemical composition of these samples, obtained by MID-FTIR spectroscopy (Picinelli and others 2006), is shown in Table 1.

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Table 1–Chemical composition of ciders.

	C3	C4	C5	C6	C9	C10	C11	C23	C24
Alcoholic degree (% v/v)	6.08	5.60	6.04	5.98	6.64	6.50	6.49	6.44	7.24
Total dry extract (g/L)	21.8	24.7	24.1	27.0	24.4	24.9	23.9	22.2	25.3
pH	3.64	3.53	3.71	3.69	3.81	3.83	3.85	3.76	3.73
Volatile acidity (g acetic/L)	0.28	0.26	1.18	0.67	1.63	0.37	1.15	1.83	1.68
Total acidity (g sulfuric/L)	2.69	3.44	4.06	3.26	3.72	2.59	3.63	4.28	3.73
Lactic acid (g/L)	5.3	6.7	6.2	6.2	5.0	5.6	5.7	6.4	4.9
1,3-Propanediol (g/L)	nd	nd	1.6	0.3	1.0	nd	1.3	2.1	0.2
Glycerol (g/L)	4.3	4.0	2.2	3.4	3.8	4.3	2.4	0.7	4.0
Sorbitol (g/L)	6.4	7.8	8.3	7.5	7.2	8.9	8.6	8.1	5.3

nd, not detected.

Table 2–References for sensory attributes of cider[a].

Attributes	Consensus definition	References
Fruity	Ripe fruit, ripe apple	Ethyl 2-methylbutyrate, 2 mg/L
Floral	Floral, roses	2-Phenylethanol, 100 mg/L
Sweet	Sweet, cake-like	Vanillin, 200 mg/L
Lactic	Lactic	Acetoin, 100 mg/L + 2-methylbutyric acid, 5mg/L

[a]Solutions prepared in ethanol:water (6:94).

Reagents and standards

The volatile standards were supplied by Sigma (St. Louis, Mo., U.S.A.), Aldrich (Gillingham, U.K.), and Flucka (Buchs, Switzerland). Pentane (VWR, Hipersolv, Darmstadt, Germany), dichloromethane (chromatographic quality), absolute ethanol, ammonium sulfate, and anhydrous sodium sulfate were from Panreac (Barcelona, Spain). All the reagents were of chromatographic quality.

Sensory analysis

Eight judges (4 men and 4 women aged from 25 to 50), belonging to the Sensory Panel of the Protected Designation of Origin "Cider from Asturias" were selected and trained for sensory descriptive analysis according to the specifications of the Spanish Assn. for Normalization (AENOR 1997). This group completed 60 h of specific training with natural ciders and references to clarify definitions, as described in Table 2.

The trained panel was scheduled to assess the samples once a week. The ciders were kept and served at 12 °C, randomly presented in normalized glasses (UNE 87021), and evaluated in duplicate. A maximum of 8 glasses were assayed in each session. Ten odor and flavor different attributes (fruity, floral, sweet, lactic, spicy, vinegar, lees, acid, bitter, and astringent) were evaluated by means of a 9-point scale (1, very weak; 5, moderate; 9, very strong). Assessments for odor, aroma, taste, and after-taste qualities were also done on a 9-point scale defined in this way: 1, very bad; 5, fair; 9, excellent.

Analysis of major volatiles compounds

Major volatiles (acetaldehyde, methanol, acrolein, ethyl acetate, 1-propanol, *iso*-butanol, 2-butanol, acetoine, amyl alcohols, allyl alcohol, ethyl lactate, and 2-phenylethanol) were analyzed by direct injection into a Hewlett-Packard model 6890 (Agilent Technologies, Palo Alto, Calif., U.S.A.) GC-FID as described elsewhere (Suárez Valles and others 2005).

Analysis of minor volatiles compounds

Minor volatile 8 alcohols (3-methyl-3-butenol, 1-pentanol, 3-methyl-2-butenol, 3-methyl-1-pentanol, hexanol, *trans*-3-hexenol, *cis*-3-hexenol, and benzyl alcohol), 5 ethyl esters (2-methylbutyrate, hexanoate, octanoate, 3-hydroxybutyrate, and 4-hydroxybutyrate), 3 fatty acids (hexanoic, octanoic, and decanoic), 2 acetate esters (isoamyl and 2-phenylethyl), 4 volatile phenols (4-ethylguaiacol, 4-ethylphenol, 4-vinylguaiacol, and 4-ethylcatechol), 3-ethoxy-1-propanol, γ-butyrolactone, and methionol were analyzed by GC-FID, after an isolation and concentration step by liquid–liquid extraction.

Cider samples were filtered through hydrophilic cotton and homogenized by magnetic stirring for 2 min. Forty-five grams of ammonium sulfate were added to a volume of 150 mL of sample, adjusting the pH to 3.00 with hydrochloric acid. Then, one-step liquid–liquid extraction was performed in 250-mL decantation funnels with 30 mL of a pentane:dichloromethane (2:1, v/v) mixture, shaking for 10 min at room temperature. Aqueous phase was discarded and then, the funnel was washed with 5 mL of the extractant mixture, and added to the organic phase. This one was centrifuged at 20000 *g* at 5 °C for 10 min. Next, 20 mL of the organic phase was collected, filtered through filter paper (Whatman 1PS silicone treated) and anhydrous sodium sulfate, washed with the extractant mixture, and subsequently concentrated by means of a Turbo Vap II^{TM} Evaporator (Zymark, Caliper Life Sciences, Mass., U.S.A.) at 30 °C under nitrogen gas flow to a final volume of 0.5 mL. Sample extracts were filtered through a 0.45 μm PVDF (polyvinyl difluorure; Teknokroma, Barcelona, Spain) before chromatographic analysis.

The chromatographic analyses were performed with a Hewlett-Packard 6890N model fitted with a flame ionization detector, a DB-WAX column (30 m × 0.32 mm., 0.50 μm, J&W Folson, Calif., U.S.A.), using helium as carrier gas (3 mL/min). Chromatographic conditions were as follows: initial temperature, 40 °C maintained for 5 min, then, program rate 3 °C/min to achieve 135 °C, 1 °C/min to reach a final temperature of 225 °C, and kept at this temperature for 5 min. Injector and detector temperatures, 275 °C. Injection volume, 1 μL (split ratio 1/50). Quantification was done by the external standard procedure. The standard solutions were prepared in a pentane:dichloromethane (2:1, v/v) mixture.

Additionally, to confirm the identity of the volatile compounds and the absence of interferences and coeluting peaks, the samples were injected onto an Agilent 7890 GC model, fitted with a mass spectrometry detector 5975C (Agilent Technologies, Palo Alto, Calif., U.S.A.), using the above-mentioned chromatographic conditions, and the total ion mode (SCAN) in the range m/z 40 to 400 (2.6 scan/s). The identification of volatile compounds was performed by comparing their spectra against the library Wiley 138, and confirmed by coinjection of the pure standards, whenever possible. Injection volume, 1 μL, was done in the splitless mode (1.0 min).

Olfactometric analysis

Olfactometric analyses were done by means of a Hewlett-Packard 5890 model fitted with a flame ionization detector and a sniffing port 275 (Ingeniería Analítica, S.L., Barcelona, Spain) heated at 220 °C, connected by a flow splitter to the exit of a

DB-WAX column (30 m × 0.32 mm; 0.50 μm from J&W Folson, Calif., U.S.A.). Gradient conditions were as follows: starting at 40 °C for 5 min, rising to 100 °C at 4 °C/min, 2nd step 6 °C/min to reach 200 °C, isocratic step at this temperature for 4 min, 10 °C/min to reach a final temperature of 225 °C, maintained for 5 min. Injector and detector temperatures, 275 °C. Injection volume 1 μL (splitless 1.0 min).

A panel of 6 to 8 people carried out the sniffings of the aforementioned cider extracts. Each judge evaluated the samples once (35 min). The overall intensity of each odor was measured using a 5-point scale (1, very weak; 3, clearly recognizable, but moderate intensity; 5, very intense odor), with 9 possible scores (half values were allowed). The data processed were a mixture of intensity and frequency of detection, named modified frequency, MF, calculated with the formula proposed by Dravnieks (1985): MF (%) $= \sqrt{F(\%)xI(\%)}$, where F(%) is the detection frequency, and I(%) the average intensity, expressed as percentage of the maximum intensity.

The identification of the odorants was carried out by comparison of their odors, by chromatographic retention index with those of the pure reference standards in both DB-WAX and DB-5 columns (30 m × 0.32 mm; 0.25 μm from J&W Folson, Calif., U.S.A.), and GC-MS spectra in the conditions established for olfactometric analyses.

Statistical analysis

To assess the influence of the cider samples, the quantitative data were submitted to variance analyses. Sensory data were evaluated by multivariate analysis of variance, taking ciders and judges as factors. To check the existence of significant differences among samples, GC/O data were submitted to 2 analyses: a χ^2 test on the citation data, and a variance analysis on the individual intensity scores. Pearson bivariate correlation analyses were also performed to seek for significant correlations between olfactometric intensities and the logarithm of concentrations of minor volatiles plus amyl alcohols and 2-phenylethanol. In all the cases, a significant level of 5% ($\alpha = 0.05$) was selected. The statistical package used was SPSS v. 12.0 for Windows (SPSS Inc., Chicago, Ill., U.S.A.).

Results and Discussion

Sensory profiles

Sensory data were evaluated by analysis of variance taking a significance level of 5% ($\alpha = 0.05$). The sensory panel disagreed in the use of the quantitative scales as the effect of judges was significant for most of the attributes; however, they were consistent at evaluating floral and lees because the interaction samples × judges were not significant (data not shown). In those cases in which significant interactions were found, a mixed model was considered, taking judges as a random effect. Thus, the F-value was calculated by dividing the mean square of cider samples by the mean square of the corresponding samples-by-judges interactions.

There were found significant differences among samples for the attributes fruity, floral, lactic, and lees. As seen in Figure 1, the samples referred to as 9, 11, and 3 had the highest scores for fruity; the cider referred to as 9 was also the most floral, sweet, and spicy, whereas sample referred to as 23 had the highest score for the attribute lees, followed by cider 10. No significant differences were found for any of the rest of the attributes. In general, these ciders were described as moderate for acid taste and weak in bitterness and astringency. It is worth to note that the vinegar sensation was assessed as weak or very weak, even though the wide range observed for volatile acidity (Table 1).

The samples were also assessed for odor and flavor quality. Pearson analyses of correlation gave several significant correlations ($P \leq 0.05$) among sensory attributes. Odor quality showed positive correlations with fruity, floral, and taste quality. In its turn, fruity was positively correlated with the attributes floral, sweet, and spicy, whereas the lactic odor exhibited negative correlations with odor and taste qualities and floral.

Quantitative aromatic profiles of ciders

The results obtained from the analyses of major volatile compounds by direct injection are summarized in Table 3. Among those compounds, it is worth to note the contents of 2-phenylethanol and amyl alcohols, with mean values of 107 and 214 mg/L respectively. Both of them are products of the alcoholic fermentation, and their contents depend on the technological conditions, such as turbidity of the musts and the making process, and the yeast strains involved in the fermentation process (Mangas and others 1993; Vidrih and others 1999; Suárez and others 2007b). Methanol, *iso*-butanol, and 1-propanol presented also significant amounts. The contents of methanol in cider are related to apple cultivar and maturity, as it is the result of pectin methyl esterase action on methoxy groups of pectins in the crushed fruit. The other 2 components are typical fermentation products, and depend on the yeast strains conducting the fermentation (Antonelli and others 1999; Satora and others 2009).

Among the minor volatile compounds, fatty acids represent 43% of the aromatic composition. Octanoic acid was the major one, followed by hexanoic and decanoic acids. The contents found in these samples were included in the range between 0.2 and 11 mg/L, with mean values similar to those described elsewhere (Mangas and others 1996; Blanco-Gomis and others 2001; Wang and others 2004). The fatty acids present in cider come from apples, their profile being related to apple cultivars and harvests (Blanco-Gomis and others 2002); alcoholic fermentation provides also a certain amount of fatty acids, which depends on the yeast strains involved in the fermentation (Arias-Abrodo and others 2005).

Volatile phenols and alcohols, respectively, account for 24% and 23% of the minor aromatic composition. Among the alcohols analyzed, hexanol was the main one, with contents ranging between 2.7 and 7.6 mg/L, similar to those reported for ciders made from single varieties (Wang and others 2004; Satora and others 2009). Other C6-alcohols analyzed were *cis*-3-hexenol and *trans*-3-hexenol, the 1st one being more important from the quantitative point of view (Table 3). Both of them had been described in apple extracts and ciders, imparting floral and herbaceous odors (Mehinagic and others 2006; Xu and others 2007). Other alcohols such as 1-pentanol and benzyl alcohol have been reported in cider extracts (Williams and May 1981).

Four volatile phenols were quantified in the ciders studied: 4-ethylguaiacol (4-EG), 4-ethylphenol (4-EP), 4-vinylguaiacol (4-VG), and 4-ethylcatechol (4-EC). Their mean contents decreased in the order 4-EC ≥ 4-EP > 4-VG > 4-EG. Comparing the values obtained for the Asturian ciders with those reported for French ciders (Buron and others 2011a), some differences can be highlighted. The Asturian ciders presented higher levels of 4-EP and lower of 4-EC than French ones. In any case, the high contents of both 4-EP and 4-EC in ciders is related to the metabolism of lactobacilli, in particular, some strains of *Lactobacillus paracollinoides*,

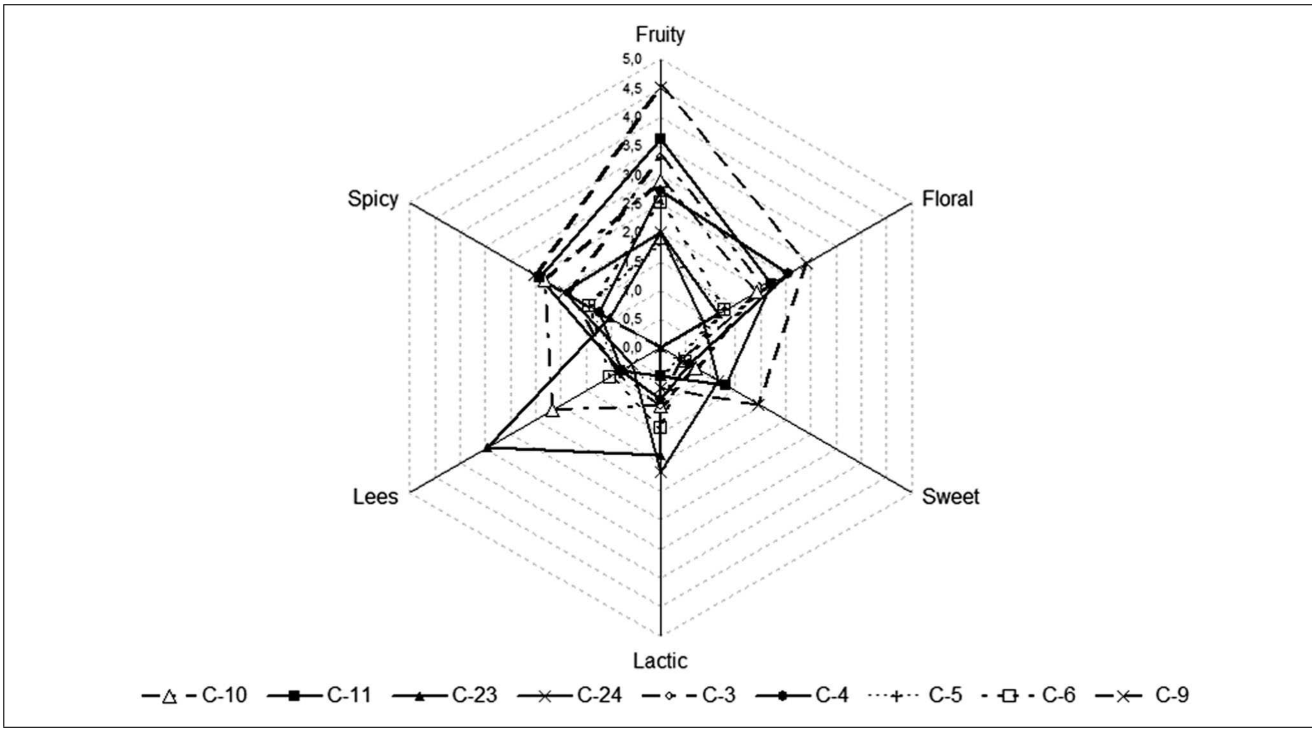

Figure 1–Spider graphs of the sensory profiles of ciders. Scores shown are mean values of the sensory panel.

which are able to synthesize those components from hydroxycinnamic acids (Buron and others 2011b).

Although volatile phenols are considered as markers of sensory defects, this concept should be revised because despite the high contents of volatile phenols found in the ciders analyzed, none of them presented this defective character. As reported elsewhere (Buron and others 2011a), a clear phenolic taint off-flavor was not perceived until a 4-EC concentration of 11.8 mg/L was achieved.

Ethyl and acetate esters represent the 5% of the minor aromatic fraction analyzed in these ciders. Among the ethyl esters, 4-hydroxybutyrate and octanoate reached the higher amounts, whereas isoamyl acetate was the major one between the analyzed acetates (Table 3). The present data are slightly lower than those observed in previous studies on Asturian ciders (Mangas and others 1996; Rodríguez Madrera and others 2005). These compounds are synthesized from alcohol and acids at different stages of the fermentation process, by the action of yeasts and lactic bacteria, or by chemical esterification during the ageing time. For instance, ethyl octanoate and ethyl hexanoate contents are influenced by the time of contact with lees (Rodríguez Madrera and others 2008). Therefore, the ester profiles are subjected to continuous changes due to the complexity of cider.

Olfactometric profiles of ciders

The results from the GC-O study of the ciders are presented in Table 4. In these ciders, 128 odorants were initially detected, but those not reaching a maximum GC-O score of 30% MF in any of the 9 ciders were not further considered. According to this criterion, the final number of components was reduced to 55, subsequently ranked and statistically evaluated. Among the components identified by olfactometry, alcohols were the most abundant, followed by ethyl esters and volatile phenols, acids, and acetate esters. All these compounds have long been reported in cider (Williams and Tucknott 1978; Williams and May 1981), cider distillates (Ledauphin and others 2003), and more recently in ciders made from Fuji (Wang and others 2004) and Chinese apple varieties (Xu and others 2007) prepared at laboratory scale, and sweet French commercial ciders (Villière and others 2012).

Two groups of odorants can be made, attending to their mean MF (%) value. In the 1st one, there were 20 components with average modified frequencies between 50% and 78%, and therefore, clearly perceived in all of the ciders; these compounds included different fermentation by-products (amyl alcohols, 2-phenylethanol, fatty acids) and volatile phenols, and they were present in a wide range of concentrations, as seen in Table 3. The highest mean value for MF corresponded to that of 4-EG and 2-methylbutyric acid (78%), followed by 2-phenylethanol (75%). In general terms, the results obtained in this set of Asturian ciders were in agreement with those reported for other ciders (Xu and others 2007); it is worth to note that many of the odorants presenting MF $\geq$ 50% in this study exhibited also the highest aroma intensities in the abovementioned report, such as ethyl esters (2-methylbutyrate, hexanoate, and octanoate), acids (2-methylbutyric, hexanoic, and octanoic), amyl alcohols and 2-phenylethanol, 2-ethylphenyl acetate, 1-octen-3-one, and volatile phenols (4-EG and 4-EP). Therefore, these compounds could be considered as part of the structural aroma of cider.

In the 2nd group, there were 35 odorants with average MF up to 46%. Again, another volatile phenol, 4-VG, reached the highest value for mean MF (46%). Most of the odorants included in this group (24) were not perceived in some of the samples, but some of them reached very high values for MF.

The ability of the odorants considered in this study to discriminate among ciders was evaluated through variance analyses of the individual intensities assigned by the participants in the sniffing sessions (ANOVA), χ^2 on the citation frequencies, and the range of the olfactometric measures (MF_{max}-MF_{min}). As shown in Table 4, 23 out of 35 components included in the 2nd group,

Table 3–Volatile composition of ciders.

	C3	C4	C5	C6	C9	C10	C11	C23	C24	Mean	SD
Major volatiles (mg/L)											
Ethyl acetate	38^{c}	17^{a}	64^{f}	45^{d}	81^{h}	33^{b}	72^{g}	55^{e}	123^{i}	59	31
Methanol	31^{c}	35^{d}	34^{cd}	17^{a}	64^{g}	28^{b}	54^{f}	44^{e}	130^{h}	49	34
1-Propanol	10^{a}	12^{b}	43^{f}	12^{b}	33^{e}	13^{b}	19^{c}	45^{g}	24^{d}	23	14
Iso-Butanol	51^{e}	32^{c}	97^{g}	25^{a}	33^{c}	45^{d}	61^{f}	28^{b}	102^{h}	53	29
Allyl alcohol	nd	nd	17^{c}	nd	4^{a}	nd	29^{d}	7^{b}	nd	6	11
1-Butanol	4^{a}	nd	nd	2^{a}	6^{c}	2^{a}	6^{bc}	5^{b}	5^{b}	3	2
Amyl alcohols	255^{g}	168^{b}	301^{h}	126^{a}	168^{b}	246^{f}	237^{e}	211^{c}	218^{d}	214	53
Ethyl lactate	231^{g}	132^{b}	166^{d}	201^{f}	188^{e}	209^{f}	117^{a}	141^{c}	258^{h}	183	47
2-Phenyl ethanol	152^{f}	17^{a}	156^{g}	16^{a}	86^{c}	137^{e}	128^{d}	244^{h}	25^{b}	107	78
Minor volatiles (μg/L)											
Fatty acids											
Hexanoic	$2{,}544^{b}$	$4{,}375^{e}$	$2{,}919^{c}$	$4{,}247^{e}$	$2{,}837^{c}$	$1{,}774^{a}$	$2{,}836^{c}$	$5{,}170^{f}$	$3{,}845^{d}$	3,394	1,074
Octanoic	$4{,}737^{de}$	$5{,}975^{f}$	$4{,}301^{cd}$	$4{,}790^{e}$	$3{,}384^{b}$	$3{,}976^{c}$	$6{,}127^{f}$	$11{,}072^{g}$	$2{,}162^{a}$	5,169	2,528
Decanoic	$1{,}581^{d}$	$1{,}058^{b}$	$1{,}211^{c}$	$1{,}011^{b}$	$1{,}333^{c}$	$1{,}591^{d}$	$2{,}271^{e}$	$4{,}331^{f}$	<320	1,626	1,151
Volatile phenols											
4-ethyl guaiacol	643^{d}	265^{b}	637^{d}	$1{,}529^{f}$	$1{,}080^{e}$	93^{a}	477^{c}	128^{a}	226^{b}	564	479
4-ethyl phenol	$1{,}371^{a}$	$2{,}509^{d}$	$1{,}723^{b}$	$2{,}856^{e}$	$3{,}181^{f}$	$1{,}616^{b}$	$2{,}308^{c}$	$2{,}926^{e}$	$1{,}317^{a}$	2,200	712
4-vinyl guaiacol	355^{bc}	$1{,}235^{e}$	609^{d}	329^{b}	411^{c}	$1{,}229^{e}$	144^{a}	$2{,}428^{f}$	307^{b}	783	733
4-ethyl catechol	504^{a}	617^{a}	586^{a}	$3{,}296^{c}$	$6{,}929^{d}$	442^{a}	$3{,}401^{c}$	$3{,}427^{c}$	$1{,}180^{b}$	2,264	2,196
Alcohols											
3-Methyl-3-butenol	<18	<18	24^{e}	18^{c}	33^{g}	<18	19^{d}	43^{h}	29^{f}	22	11
1-Pentanol	45^{c}	37^{b}	54^{d}	39^{b}	110^{e}	35^{b}	<12	219^{f}	108^{e}	72	65
3-Methyl-2-butenol	14^{b}	11^{a}	41^{e}	17^{c}	41^{e}	17^{c}	34^{d}	72^{f}	33^{d}	31	19
3-Methyl-1-pentanol	43^{e}	8	35^{d}	9^{a}	41^{e}	23^{c}	48^{f}	93^{g}	20^{b}	36	26
Hexanol	$3{,}646^{b}$	$3{,}910^{bc}$	$4{,}394^{cd}$	$5{,}736^{f}$	$5{,}266^{ef}$	$2{,}746^{a}$	$4{,}245^{c}$	$7{,}638^{g}$	$4{,}831^{de}$	4,712	1,409
trans-3-Hexenol	23^{b}	41^{f}	34^{d}	37^{e}	30^{c}	18^{a}	40^{f}	51^{g}	34^{d}	34	10
cis-3-Hexenol	421^{f}	103^{a}	274^{d}	341^{e}	177^{b}	216^{c}	185^{b}	548^{g}	190^{b}	273	141
Benzyl alcohol	64^{ab}	55^{a}	321^{g}	78^{b}	267^{f}	141^{c}	219^{d}	374^{h}	240^{e}	195	117
Ethyl esters											
2-Methylbutyrate	9^{ab}	7^{a}	11^{b}	23^{c}	30^{d}	10^{b}	29^{d}	35^{e}	33^{e}	21	11
Hexanoate	105^{b}	204^{f}	131^{c}	229^{g}	132^{c}	91^{a}	148^{d}	217^{fg}	168^{e}	158	49
Octanoate	158^{d}	181^{e}	172^{e}	172^{e}	144^{c}	132^{b}	203^{f}	324^{g}	102^{a}	176	63
3-Hydroxybutyrate	18^{b}	8^{a}	14^{b}	44^{c}	124^{f}	18^{b}	68^{d}	99^{e}	120^{f}	57	47
4-Hydroxybutyrate*	239^{c}	262^{d}	446^{f}	283^{d}	610^{g}	174^{a}	377^{e}	379^{e}	217^{b}	332	136
Acetate esters											
Isoamyl	428^{e}	389^{d}	472^{f}	77^{a}	199^{b}	396^{d}	370^{d}	528^{g}	314^{c}	353	139
2-Phenyl ethyl	478^{h}	41^{d}	167^{f}	<18	84^{e}	55^{cd}	72^{de}	208^{g}	24^{b}	126	147
Others											
3-Ethoxy-1-propanol	39^{a}	35^{a}	312^{e}	84^{b}	179^{c}	97^{b}	748^{g}	364^{f}	252^{d}	234	226
γ-Butyrolactone	$1{,}879^{d}$	$3{,}864^{f}$	$2{,}567^{e}$	$3{,}804^{f}$	$1{,}485^{c}$	$1{,}008^{b}$	850^{b}	361^{a}	$1{,}437^{c}$	1,917	1,254
Methionol	557^{c}	622^{d}	$1{,}012^{h}$	418^{b}	360^{a}	710^{e}	363^{a}	933^{g}	785^{f}	640	240

nd, not detected; *quantified as ethyl 3-hydroxybutyrate. Values with different letters in the same column are significantly different ($\alpha = 0.05$).

and 5 out 20 of those present in the 1st one, were found to be potentially differencing, as measured by both statistical tests. The χ^2 showed significant differences for other 5 compounds (4-EG, sotolon, *p*-cresol, *m*-cresol, and γ-decalactone). Likewise, all the above potentially discriminatory compounds exhibited ranges of their modified frequencies higher or equal to 30%, excepting 4-EG. However, the opposite was not always true since 10 components, which are written in bold letters in Table 4, did not significantly discriminate among ciders, despite their high ranges for MF (%). The intrinsic properties of the components analyzed may explain some of the difficulties found to discriminate between stimulus levels. In the case of methionol, its olfactory signal becomes saturated at concentrations only 10-fold its perception threshold (Culleré and others 2004). The opposite situation was represented by ethyl propionate, isoamyl acetate, and hexanol, producing transitory stimulus, with low to medium olfactory intensities.

In order to identify the most powerful odorants, the cider referred to as C3 was selected on the basis of its high odor intensity and quality (data not shown) to perform an AEDA study. To do so, the extract was sequentially diluted by a rate of 2 with the extractant mixture, analyzed by the 5 most sensitive judges, and the citation frequencies being measured. The flavor dilution factor of each odorant corresponds to the maximum dilution that could be perceived by at least one of the panelists, and it is shown in the last column of Table 4.

According to the results of this experiment, 28 components had values for DF different from zero. The most powerful odorants were 2-phenylethanol, 4-EG and 4-EP, followed by amyl alcohols, *trans*-3-hexenol, 2-methylbutyric acid, and octanoic acid. Other odorants such as sotolon, methionol, ethyl octanoate, 3-methyl-2-butenol, and 4-VG had also noticeable values for dilution factors. Taking apart 2-phenylethanol and amyl alcohols, being major components of the volatile profile of cider, the other compounds can be considered as potentially active odorants of this beverage. Likewise, it is worth to note that 4 of these potentially active compounds were also identified as discriminatory: 4-EG, octanoic acid, ethyl octanoate, and 4-VG (Table 4).

Table 4–Olfactometric profiles of ciders: olfactory description, chemical identification, modified frequencies, MF (%), and AEDA dilution factors (DF).

LRI_{WAX}	LRI_5	Description	Chemical identity	C3	C4	C5	C6	C9	C10	C11	C23	C24	Mean	χ^2	ANOVA	difMax-min	DF
Average MF ≥ 50%																	
931	721	Fruity	Propyl acetate	64	62	56	54	53	59	51	47	68	57	ns	ns	21	16
1074	805	Fruity, sweet, apple	Ethyl 2-methyl butyrate	69	46	55	65	48	68	70	75	75	63	ns	ns	28	4
1217	753	Like amylics	Amyl alcohols	69	76	75	65	66	65	72	58	65	68	ns	ns	18	128
1240	1186	Ripen fruit	**Ethyl hexanoate**	65	44	37	56	45	52	52	35	59	50	ns	ns	30	0
1294	983	Mushroom	1-Octen-3-one	63	43	32	48	72	47	53	70	72	55	**	**	40	32
1299	805	Smoked, toasted	**3-Methyl-2-butenol**	40	69	59	48	26	65	68	51	58	54	ns	ns	43	32
1383	887	Flowery, geranium	*trans*-3-Hexenol	75	74	76	69	73	71	68	72	80	73	ns	ns	12	128
1448		Vinegar, like fusel alcohols, ketchup	**Acetic acid**	53	40	65	56	61	56	74	56	65	58	ns	ns	33	0
1635	954	Rancid, varnish	γ-Butyrolactone	75	80	61	77	77	76	81	72	67	74	ns	ns	20	16
1681		Cheese, rancid	2-Methyl butyric acid	84	73	76	77	79	83	76	79	77	78	ns	ns	11	128
1837	1267	Stewed fruit, flowery	2-Phenylethyl acetate	69	45	74	55	53	56	56	69	49	59	ns	ns	29	16
1860	1174	Fatty, stable	Hexanoic acid	70	71	76	66	67	76	73	73	83	73	ns	ns	16	16
1903	1071	Fruity, flowery, sweet	Benzyl alcohol	47	16	66	51	64	52	40	56	64	50	**	**	50	8
1929	1175	Roses	2-Phenyl ethanol	59	78	76	84	82	82	74	63	81	75	ns	ns	25	256
2047	1294	Sweet, spicy	4-Ethyl guaiacol	77	77	75	76	77	77	78	85	77	78	**	ns	10	256
2078	1358	Fatty, stable, spicy	Octanoic acid	78	58	62	65	59	81	78	73	63	69	**	**	23	128
2153		Leather, smoked	ni	79	40	47	43	67	41	47	47	76	54	**	**	39	16
2194	1165	Leather, stable	4-Ethyl phenol	70	76	65	57	76	65	77	69	62	68	ns	ns	20	256
2210	1095	Smoked, spicy, curry	Sotolon	65	85	61	77	46	55	81	85	72	70	**	ns	39	64
2285	1451	Fatty, stable	Decanoic acid	68	65	35	55	36	77	73	77	45	59	**	**	42	16
Average MF < 50%																	
905	719	Fruity	**Ethyl propionate**	9	0	34	13	27	16	18	28	6	17	ns	ns	34	0
1057	804	Fruity	Ethyl butyrate	38	44	7	44	42	26	38	48	47	37	**	**	41	16
1093	818	Ripen fruit	Butyl acetate	31	44	0	27	13	0	0	6	0	14	**	**	44	0
1144	879	Fruity, banana	**Isoamyl acetate**	20	13	29	0	16	25	24	16	30	19	ns	ns	30	0
1364	889	Flowery, fruity	**Hexanol**	43	21	47	46	37	52	18	27	39	37	ns	ns	33	0
1404	867	Flowery, sweet	*cis*-3-Hexenol	49	9	7	30	0	36	29	53	0	24	**	**	53	0
1413		Vinegar, like fusel alcohols	ni	0	0	0	0	22	36	0	0	0	7	**	**	36	0
1419		Sweet, spicy	ni	0	0	7	0	0	0	0	0	65	8	**	**	65	0
1432	1016	Sweet, spicy, fruity	2-Octanol	29	6	0	9	34	0	0	29	0	12	**	**	34	0
1440	1190	Fruity, grass, resinous	Ethyl octanoate	52	60	17	63	6	40	29	27	24	35	**	**	57	32
1466	908	Dust, rancid, cooked vegetables	Methional	0	49	0	0	25	9	9	0	9	11	**	**	49	0
1486		Fruity, flowery, sweet	ni	0	0	0	0	47	6	34	16	20	14	**	**	47	0
1494	957	Fruity, flowery	Ethyl 3-hydroxybutyrate	36	25	21	18	27	38	36	29	33	29	ns	ns	20	8
1519	856	Fatty, stable	Propanoic acid	0	0	68	0	0	0	0	57	0	14	**	**	68	0
1529	909	Fatty, heavy	*iso*-Butyric acid	6	0	32	22	60	29	0	40	51	27	**	**	60	0
1554	1096	Fatty, resinous	1-Octanol	59	17	32	0	19	20	9	0	0	17	**	**	59	0
1567	966	Lactic, sharp, blue cheese	Butanoic acid	45	38	45	6	0	17	22	9	55	26	**	**	55	0
1653	1090	Flowery	Phenyl acetaldehyde	77	6	65	13	46	30	47	58	19	40	**	**	71	0
1735	1013	Cooked vegetables, sulfury	**Methionol**	38	60	42	57	16	59	34	48	43	44	ns	ns	44	64
1775		Flowery, fruity, coconut	ni	49	13	15	13	49	0	29	40	53	29	**	**	53	2
1801		Sweet flowery	Ethyl 4-hydroxybutyrate[a]	22	16	34	6	6	13	13	13	13	15	ns	ns	28	2
1880	1118	Smoked, sweet, spicy	**Guaiacol**	37	39	39	40	38	40	9	25	43	35	ns	ns	34	8
1986		Flowery, spicy	**ni**	44	6	45	19	0	29	27	29	34	26	ns	ns	45	0
2001	1088	Smoked	***o*-Cresol**	29	26	42	21	53	41	29	30	20	32	ns	ns	33	0
2100	1123	Leather, stable	*p*-Cresol	0	0	47	29	17	26	27	43	23	24	**	ns	47	0
2109	1099	Leather, stable	*m*-Cresol	40	30	24	47	10	23	18	0	28	25	**	ns	47	8
2171	1476	Spicy, sweet, leather	γ-Decalactone	0	9	34	70	60	51	40	73	29	41	**	ns	73	0

Continued

Table 4–Continued.

LRI_{WAX}	LRI_5	Description	Chemical identity	C3	C4	C5	C6	C9	C10	C11	C23	C24	Mean	χ^2	ANOVA	difMax-min	DF
2183	1385	Spicy, clover	Eugenol	70	76	54	0	41	65	0	0	62	41	**	**	76	0
2219		Smoked	ni	0	0	0	9	40	60	0	0	0	12	**	**	60	0
2246	1279	Smoked, spicy	4-Vinyl guaiacol	63	34	29	62	61	37	53	49	26	46	**	**	37	32
2261	1913	Resinous, dry grass	Ethyl hexadecanoate	0	0	0	0	0	36	6	34	38	13	**	**	38	0
2305		Flowery, vegetal	ni	0	0	0	0	33	6	18	9	36	11	**	**	36	0
2323		Balsamic, sweet	ni	6	0	0	0	0	0	31	27	40	12	**	**	40	16
2333		Stable, phenolic	ni	11	6	7	42	65	31	54	56	35	34	**	**	58	0
2360	1496	Spicy, phenolic	Isoeugenol	45	51	0	19	9	6	6	0	24	18	**	**	51	4

LRI_{WAX}, LRI_5: linear retention index on DB-WAX and DB-5 columns, respectively; ni: not identified; ns: not significant
[a]significant at the 5% level.

Table 5–Pearson correlation coefficients between odor sensory attributes and olfactometric mean intensities or logarithm of volatile contents.

Odor attributes	I (%)	Log concentration
Vinegar	Butyl acetate (−0.706; $P = 0.034$)	–
	Amyl alcohols (−0.679; $P = 0.044$)	
	LRI 1413 (0.755; $P = 0.019$)	
	LRI 2219 (0.767; $P = 0.016$)	
	Isoeugenol (−0.739; $P = 0.023$)	
Fruity	*t*-3-hexenol (−0.677; $P = 0.045$)	Methionol (−0.831; $P = 0.005$)
	LRI 1486 (0.626; $P = 0.071$)	
	Methionol (−0.745; $P = 0.021$)	
Sweet	LRI 1486 (0.842; $P = 0.004$)	Methionol (−0.659; $P = 0.053$)
	Methionol (−0.761; $P = 0.017$)	
	4-EP (0.819; $P = 0.007$)	
	LRI 2305 (0.814; $P = 0.008$)	
Spicy	Amyl alcohols (−0.663; $P = 0.051$)	Methionol (−0.608; $P = 0.082$)
	LRI 1486 (0.767; $P = 0.016$)	
	Methionol (−0.772; $P = 0.015$)	
	4-EP (0.837; $P = 0.005$)	
	Sotolon (−0.635; $P = 0.066$)	
	LRI 2305 (0.728; $P = 0.026$)	
Lees	Ethyl 2-methylbutyrate (0.673; $P = 0.047$)	Octanoic acid (0.671; $P = 0.048$)
		Decanoic acid (0.602; $P = 0.087$)
	2-Methylbutyric acid (0.642; $P = 0.062$)	4-EG (−0.682; $P = 0.043$)
		4-VG (0.725; $P = 0.027$)
	4-EP (−0.627; $P = 0.071$)	γ-butyrolactone (−0.668; $P = 0.049$)
	Ethyl hexadecanoate (0.634; $P = 0.067$)	

The role that some of these potentially active components may play in the interpretation of sensory perceptions can be evaluated through the correlations obtained between the mean scores of sensory attributes and the mean olfactometric intensities or the logarithm of volatile contents, as shown in Table 5.

Thus, the olfactometric intensities of 4-EP, amyl alcohols, *trans*-3-hexenol, 2-methylbutyric acid, methionol, and sotolon were significantly correlated with different odor sensory attributes. Other components such as butyl acetate or the nonidentified peaks (LRI 1413, 1486, and 2305) showed also significant correlations with sensory descriptors.

In agreement with their respective olfactometric assessments, the vinegar odor was negatively correlated with butyl acetate, and positively related to the nonidentified peak at 1413, described as vinegar (Table 4). The sensory attributes fruity, sweet, and spicy were positively correlated with the unknown peak at 1486 (described as fruity, floral, and sweet), and negatively with methionol (Table 5). The powerful odorant 4-EP was directly associated with the sensory perceptions sweet and spicy, and inversely with the attribute lees.

The quantitative volatile composition analyzed gave less significant correlations with the sensory descriptors of ciders. It is worth to note that no relationship was found between the vinegar odor and the volatile acidity of the ciders. In fact, values for this parameter higher than 1.40 g acetic/L are common in Asturian Cider (Picinelli and others 2000) with no detrimental effect on its sensory quality. As reported elsewhere, acetic acid is more penetrating in thin ciders of low acidity than in full-bodied ones (Whiting 1973). Similarly, the odor detection for acetic acid in Ice wines is 3-fold that of table ones (Cliff and Pickering 2006), reinforcing the hypothesis of the masking effect of cider matrix. Likewise, methionol content was negatively correlated with the assessments of fruity, sweet, and spicy, while lees was directly associated to fatty acids and 4-VG, and inversely with 4-EG and γ-butyrolactone.

Unlike previous results on wine aroma (Escudero and others 2007), no correlations were found between fruity sensory notes and the contents of ethyl or acetate esters in cider, neither individually nor grouped (data not shown). Compared with wine aromatic composition, fatty acids and volatile phenols are predominant in cider, so that a strong masking effect should be expected. Also, many other cider components of cider such as polyphenols could contribute to decrease the volatility of those esters and subsequently, their sensory perception (Lorrain and others 2013).

Conclusions

The quantitative analysis of these ciders has shown the presence of high amounts of fatty acids, alcohols, and volatile phenols as typical components of the aromatic profile of quality ciders. From the olfactometric study carried out, 55 components have been ranked by their intensities, 28 of them were found to be of greater importance to discriminate among samples. Components such as amyl alcohols, 2-phenylethanol, ethyl esters such as 2-methylbutyrate, hexanoate and octanoate, hexanoic and octanoic acids 2-phenylethyl acetate, 4-EG and 4-EP, were found to be common to other ciders, so that they could be considered as part of the aromatic structure of this beverage. Two volatile phenols (4-EG and 4-EP) were identified as powerful odorants, and were involved in the perception of the sensory attributes sweet, spicy, and lees.

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