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Characterization of Spanish ciders by means of chemical and olfactometric profiles and chemometrics

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ABSTRACT

A comparative study of the aroma (volatile composition and olfactometric profiles) of Asturian and Basque still ciders in two maturation stages was conducted. Among the major volatile compounds, amyl alcohols, ethyl lactate and ethyl acetate were quantitatively relevant in all of the ciders studied. The minor fraction mainly consisted of fatty acids, volatile phenols and alcohols. Three PLS-discriminant models with low prediction errors were constructed. When the volatile composition was used, ciders could be differentiated by their maturation stage, 4-ethylcatechol being strongly associated to matured ciders. The olfactometric profiles allowed the classification of ciders according to both their origin and maturation stage. Odorants such as *p*-cresol and a sweet-character unknown component were correlated to origin of ciders, whereas 1-octen-3-one and one unknown spicy-vegetal odorant were highly correlated to the maturation stage.

1. Introduction

Cider is a worldwide popular drink made by fermentation of apple juices without at any time adding distilled alcohol (www.aicv.org). The consumption rates of this beverage have achieved a significant rise in recent years; moreover, the global market potential of cider is projected to reach 12.9 billion dollars by 2020 (www.strategyr.com/MarketResearch). The term "cider" includes both innovative products, such as new flavoured ciders (mixtures of apple with berries, passion fruit, kiwi, lime), and artisanal presentations strongly associated to a given geographical area, elaborated according to *traditional good manufacturing practices*. The European Union food quality policy encourages the protection of food according to specific schemes (Protected Designation of Origin (PDO), Protected Geographical Indication (PGI)) in order to guarantee quality to consumers and a fair price for producers. Therefore, objective tools to demonstrate the tipycity and excellence of protected foods are needed.

Nowadays, different spectroscopical and chromatographic methodologies based on the profiling of trace elements, isotope ratios, phenolic and volatile compounds, in combination with chemometric techniques, have been proposed for geographical discrimination and product characterisation, as recently reviewed (Cubero-León, Peñalver, & Maquet, 2014; Versari, Laurie, Ricci, Laghi, & Parpinello, 2014).

Asturias is the leading producer of cider in Spain, followed by the Basque Country. Asturian Cider is included as a PDO in the register of the European Commission (EC 2154/2005) and therefore, the interest in characterizing its chemical and sensory attributes is evident. Aroma of ciders can be influenced by several factors. Apple varieties, pressing systems, the microorganisms involved in the fermentation process, and ageing on lees are paramount to define the volatile profile of ciders (Antón-Díaz, Suárez Valles, Mangas-Alonso, Fernández-García, & Picinelli-Lobo, 2016; del Campo et al., 2003; Le Quéré, Husson, Renard, & Primault, 2006; Mangas, González, & Blanco, 1993; Satora et al., 2009; Suárez Valles, Pando Bedriñana, Fernández Tascón, González García, & Rodríguez Madrera, 2005; Suárez Valles, Pando Bedriñana, Fernández Tascón, Querol Simón, & Rodríguez Madrera, 2007). Minor components such as fatty acids and esters allowed to discriminate ciders on the basis of the harvest (Blanco-Gomis, Mangas-Alonso, Margolles-Cabrales, & Arias Abrodo, 2001; Rodríguez Madrera, Palacios García, García Hevia, & Suárez Valles, 2005), whereas sweet ciders from Normandy and Brittany (France) could be differentiated by their contents in isoamyl acetate and hexyl acetate (Haider, Barilier, Hayat, Gaillard, & Ledauphin, 2014).

In this paper, the aromatic profiles (chemical and olfactometric) of the main Spanish ciders are described and evaluated as potential criterion for discrimination by origin or maturation stage.

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2. Materials and methods

2.1. Cider samples

A total of 58 still natural ciders from Asturias (n = 38) and Basque Country (n = 20) were sampled for analysis at bottling from their respective cellars. In each case, ciders were categorized into "Young" (n = 30), as those samples collected at a maximum ageing of six months since the pressing time, and "Matured" (n = 28), or aged samples. These ciders were made according to the traditional methods used in each region, by spontaneous fermentation of complex apple mixtures, and contact with lees. Their oenological characteristics are summarized in Table 1. These samples were kept at 12 °C until chemical and olfactometric analysis for a maximum of one month.

2.2. Reagents and Standards

The volatile standards were supplied by Sigma (St. Louis, MO), Aldrich (Gillingham, U.K.) and Fluka (Buchs, Switzerland). Dichloromethane, absolute ethanol, ammonium sulphate and anhydrous sodium sulphate were from Panreac (Barcelona, Spain), and pentane from VWR (Darmstad, Germany). All the reagents were of chromatographic quality.

2.3. Analysis of major volatiles compounds

Acetaldehyde, methanol, acrolein, ethyl acetate, 1-propanol, isobutanol, 2-butanol, acetone, amyl alcohols (sum of isoamyl and amyl), allyl alcohol, ethyl lactate, and 2-phenylethanol were analyzed by direct injection into a Hewlett Packard model 6890N (Agilent Technologies, Palo Alto, CA, USA) GC-FID as described elsewhere (Suárez Valles et al., 2005).

2.4. Analysis of minor volatiles compounds

Eight 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), seven ethyl esters (propionate, butyrate, 2-methylbutyrate, hexanoate, octanoate, 3-hydroxybutyrate and 4-hydroxybutyrate), three fatty acids (hexanoic, octanoic and decanoic), four acetate esters (propyl, butyl, isoamyl and 2-phenylethyl), four 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 with a mixture of pentane:dichloromethane (2:1), as described by Antón, Suárez Valles, García Hevia, & Picinelli Lobo, 2014.

2.5. Olfactometric analysis

Olfactometric analyses were done by means of a Hewlett-Packard 5890 model fitted with a flame ionization detector, coupled to an olfactory port at 220 °C (model 275, Ingeniería Analítica, S.L., Barcelona, Spain), and a DB-WAX column (30 m × 0.32 mm; 0.50 µm from J&W Folson, CA, USA). The identification of the odorants was done by comparing their odour profiles and retention indexes with those of the pure reference standards, whenever possible, in both DB-WAX and DB-5 (30 m × 0.32 mm; 0.25 µm from J&W Folson, CA, USA) columns, and compared to those found in the literature (Table 2). GC–MS spectra were also obtained by injecting onto an Agilent 7890 GC model (Agilent Technologies, Palo Alto CA, USA), fitted with a mass spectrometry detector 5975C running in the total ion mode (SCAN) in the range m/z 40–400 (2.6 scan/s). A panel of 6–8 people carried out the sniffings of the aforementioned cider extracts. Panelists were trained for odour recognition (4 sessions), with synthetic solutions in EtOH 6% (v/v). Subsequently, they were instructed in the olfactometric technique by using synthetic mixtures of increasing concentrations (5 sessions), and finally, 2 sessions with real samples to check their ability to perform the analysis and reproducibility. Each judge evaluate the samples once (35 min), using a five-point intensity scale, as described elsewhere (Antón et al., 2014). Data were expressed in terms of mean intensities (I,%).

2.6. Statistical analysis

Multivariate analysis of variance was done to check the influence of origin or maturation on the oenological parameters of cider. Non-parametric χ^2 tests were done to assess the effect of these factors on the chemical and olfactometric profiles by using the statistical package SPSS v.12 for Windows. Subsequently, discriminant-PLS multivariate analyses were performed for modeling ciders according to their origin by defining the following binary response variable: Y = 0, Asturian ciders; Y = 1, Basque ciders. For maturation models, the binary response variable was defined as Y = 0, Matured ciders; Y = 1, Young ciders. Predictor variables were the olfactometric peaks (57) or the volatile components (41). The discriminant models were validated by cross-validation with three cancellation groups, by randomly assigning nineteen samples in

Table 1
Oenological parameters, non-volatile acids, fructose and polyalcohol contents in Spanish natural still ciders (mean, SD, maximum and minimum values).

				Asturias								Basque country							
	Significance			Young				Matured				Young				Matured			
	Int	Or	Mat	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Alcoholic degree (% v/v)	**	**	ns	6.83	0.43	7.46	5.57	6.61	0.16	6.92	6.38	6.62	0.25	7.08	6.23	6.56	0.35	7.12	6.09
pH	ns	***	***	3.82	0.08	3.93	3.60	3.87	0.07	3.97	3.72	3.68	0.08	3.81	3.55	3.77	0.06	3.87	3.67
Volatile acidity (g acetic acid/L)	ns	**	***	0.98	0.53	1.88	0.19	2.24	0.46	2.90	1.32	0.94	0.35	1.28	0.27	1.81	0.55	2.20	1.06
Total acidity (g sulfuric acid/L)	ns	ns	***	3.25	0.46	3.82	2.18	3.93	0.39	4.78	3.19	3.45	0.21	3.84	3.14	3.99	0.47	4.99	3.40
Lactic acid (g/L)	ns	ns	ns	4.8	0.9	7.9	3.7	4.5	0.7	6.6	3.3	4.8	0.6	5.7	4.2	4.9	0.7	6.0	3.6
Acetic acid (g/L)	ns	ns	***	0.97	0.50	1.90	0.30	1.90	0.19	2.10	1.40	1.00	0.34	1.40	0.30	1.60	0.30	2.00	1.09
Glycerol (g/L)	ns	ns	***	3.1	1.2	5.3	0.1	1.5	1.1	3.7	0.1	3.5	1.0	4.6	1.4	1.7	0.9	3.3	0.4
Sorbitol (g/L)	ns	***	ns	7.1	1.7	11.6	4.2	5.8	0.7	7.1	4.6	5.6	0.9	7.6	4.8	5.0	1.2	7.5	3.6

Int: interaction; Or: origin; Mat: maturation; SD: standard deviation; Max: maximum; Min: minimum; ns: not significant.

** Significant at $p < 0.05$.

*** Significant at $p < 0.01$.

Table 2
Comparison between the linear retention indexes calculated in the olfactometric conditions and those found in the literature.

Values found in the literature[1,2,3]					
LRI_{WAX}	LRI_{DB5}	LRI_{WAX}	Description	Identity	RefV
905	719	950–959	Fruity	Ethyl propionate	v1
1057	804	1028–1054	Fruity	Ethyl butyrate	v4
1074	805	1045–1067	Fruity, apple-like	Ethyl 2-methylbutyrate	v5
1144	879	1102–1137	Fruity, banana	Isoamyl acetate	v7
1217	753	1201–1223	Like fusel alcohols	Amyl alcohols	v8
1240	1183	1229–1246	Ripen fruit	Ethyl hexanoate	v10
1294	983	1301–1307	Mushroom	1-Octen-3-one	v12
1364	889	1354	Herbaceous, floral	Hexanol	v15
1383	887	1379–1386	Floral, geranium	*t*-3-Hexenol	v16
1404	867	1396	Floral	*c*-3-Hexenol	v17
1440	1190	1409–1427	Fruity, resin	Ethyl octanoate	v21
1448		1434–1459	Vinegar-like, fusel alcohols	Acetic acid	v22
1466	908	1463	Dust, rancid, cooked vegetables	Methional	v23
1494	957	1501	Fruity, floral	Ethyl 3-hydroxybutyrate	v27
1519	856	1523	Fatty, stable	Propanoic acid	v29
1635	954	1635–1663	Rancid, varnish	γ-Butyrolactone	v34
1681		1660–1692	Cheese, rancid	2-Methylbutyric acid	v36
1735	1013	1695–1714	Cabbage	Methionol	v38
1837	1267	1801–1859	Stewed fruit, floral	2-Phenylethyl acetate	v43
1860	1174	1840–1846	Fatty, stable	Hexanoic acid	v44
1880	1118	1882	Sweet, smoky, spicy	Guaiacol	v45
1929	1175	1906–1968	Roses	2-Phenylethanol	v47
2047	1294	2024–2063	Sweet spicy	4-Ethylguaiacol	v52
2078	1358	2058–2060	Fatty, sweat	Octanoic acid	v54
2100	1123	2087	Leather, stable	*p*-Cresol	v57
2183	1385	2164–2171	Spicy, clove	Eugenol	v63
2194	1165	2181–2195	Leather, stable	4-Ethylphenol	v64
2210	1095	2240	Smoky, spicy, curry	Sotolon	V66
2246	1279	2200–2247	Spicy	4-Vinylguaiacol	v68
2285	1451	2272–2282	Fatty, sweat	Decanoic acid	v71
2360	1496	2394	Smoky, spicy	Isoeugenol	v77

[1] Xu, Fan, and Qian (2007).
[2] Villière et al., (2012).
[3] Campo, Cacho, and Ferreira (2008).

each group. These modeling analyses were performed with the Unscrambler v.9.2 statistical package.

3. Results and discussion

3.1. Chemical composition of ciders

Table 1 describes the enological characteristics of ciders. The Spanish still cider is a dry and acidic beverage, with an alcoholic degree between 5.5 and 7.5 (% v/v) depending on the characteristics of the raw material. Its non-volatile composition is dominated by polyalcohols such as glycerol and sorbitol, and organic acids such as lactic and acetic. Origin of ciders significantly influenced pH, volatile acidity and contents of sorbitol, whereas cider maturity affected all of these parameters, with the exception of lactic acid and sorbitol levels.

Cider-making could explain some of the special features of the chemical composition of Spanish still ciders shown in Table 1. Sugars and malic acid are absent, as both alcoholic and malolactic fermentations are completed. Also, it could be mentioned the relatively high values for volatile acidity, comparing with other beverages. This fact is not always objectionable to sensory quality (Antón et al., 2014; Picinelli et al., 2000), but appreciated because of the positive influence of volatile acidity on the sensory foaming assessment of cider (Mangas, Moreno, Rodríguez, Picinelli, & Suárez, 1999).

3.2. Quantitative volatile profiles

A descriptive summary of the volatile composition of ciders is presented in Table 3. As it can be seen, the major volatile fraction is dominated by amyl alcohols, ethyl lactate and 2-phenylethanol, whereas fatty acids, volatile phenols and alcohols were the most important families among the minor components. Their presence and contents were similar to those found in other fermented beverages, as they are typical products of the fermentation process (Pires, Teixeira, Brányik, & Vicente, 2014; Swiegers, Bartowsky, Henschke, & Pretorius, 2005). Mean contents of fatty acids and volatile phenols were consistent with those previously reported in ciders (Blanco-Gomis et al., 2001; Buron, Guichard, Coton, Ledauphin, & Barillier, 2011a; Haider et al., 2014; Wang, Xu, Zhao, & Li, 2004) but higher than those found in red wines, especially with regard to volatile phenols (San Juan, Cacho, Ferreira, & Escudero, 2012).

The origin of ciders significantly influenced the contents of 1-butanol, 2-phenylethanol, *c*-3-hexenol, two ethyl esters (octanoate and 4-hydroxybutyrate) and three acetates (butyl, isoamyl and 2-phenylethyl). All of these components presented higher concentrations in the Asturian ciders excepting1-butanol. Those differences could be attributed to diverse technological factors: apple mixtures (del Campo, Berregi, Santos, Dueñas, & Irastorza, 2008; Villière, Arvisenet, Bauduin, Le Quéré, & Sérot, 2015), pressing and/or clarification systems (del Campo et al., 2003; Mangas et al., 1993; Satora et al., 2009; Villière et al., 2015), and the microorganisms (yeasts and lactic bacteria strains) involved in the fermentation process (Suárez Valles et al., 2005). The fermentation step (alcoholic and malolactic transformation) takes place spontaneously in the making of both Asturian and Basque ciders and therefore, a great diversity of yeast species are present throughout the making process (Pando Bedriñana, Querol Simón, & Suárez Valles, 2010; Suárez Valles et al., 2007).

The high contents of 2-phenylethanol reported in these natural ciders, particularly in those from Asturias, could be associated with

Table 3
Quantitative aromatic composition of Spanish natural still ciders (mean, standard deviation, maximum and minimum values).

	Significance		Asturias								Basque country							
			Young				Matured				Young				Matured			
	Origin	Mat	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Major volatiles (mg/L)																		
Acetaldehyde	ns	***	1	1	6	nd	4	6	17	nd	nd				2	4	12	nd
Ethyl acetate	**	***	46	25	102	18	93	35	172	38	30	10	48	17	67	21	97	30
Methanol	ns	***	36	16	69	5	81	41	214	33	37	14	58	19	63	11	80	49
2-Butanol	ns	***	3	6	22	nd	18	15	55	nd	1	1	3	nd	9	8	29	1
1-Propanol	ns	***	12	10	42	2	42	20	77	9	7	3	10	3	47	30	115	15
i-Butanol	**	*	37	18	90	11	39	8	62	24	26	7	38	15	37	19	82	15
Allyl alcohol	**	***	4	6	20	nd	9	7	30	nd	nd		2	nd	7	9	27	1
1-Butanol	**	ns	4	1	6	2	5	1	7	4	6	2	10	3	6	1	8	4
Amyl alcohols[a]	***	***	171	48	283	95	193	32	248	125	122	24	176	102	179	68	362	125
Acetoine	ns	ns	3	2	12	nd	3	2	7	nd	2	1	5	nd	3	1	5	nd
Ethyl lactate	ns	***	113	56	226	43	192	42	284	111	122	45	168	38	218	35	294	171
2-Phenyl ethanol	***	ns	93	52	200	15	95	38	178	35	42	33	123	15	62	50	181	26
Minor volatiles (μg/L)																		
Fatty acids																		
Hexanoic	*	***	2638	859	5783	1609	3802	1762	9416	2239	2711	888	4395	1572	4477	1234	6840	2437
Octanoic	ns	ns	4344	1624	8752	2361	4824	2982	13,727	2367	3409	1021	5106	2028	5241	1882	8455	2991
Decanoic	ns	**	2297	709	3619	1182	2066	1352	5947	916	1717	672	2795	452	1793	514	2731	970
Volatile phenols																		
4-Ethylguaiacol	ns	ns	582	282	1036	<QL	809	496	2688	353	639	352	1304	<QL	533	290	942	87
4-Ethylphenol	ns	*	1972	574	3080	1105	2774	1481	8165	1743	2243	1020	4819	904	2310	416	3281	1848
4-Vinylguaiacol	***	***	2221	842	3922	920	834	214	1509	450	1121	544	1967	232	nd	nd	nd	nd
4-Ethylcatechol	ns	***	2643	2094	9711	286	5167	2158	8713	1256	2276	1896	5891	<QL	5506	1972	9195	2130
Alcohols																		
3-Methyl-3-butenol	ns	***	26	7	39	<QL	47	18	95	29	27	12	44	<QL	31	8	46	<QL
1-Pentanol	ns	***	70	16	94	42	145	63	334	84	93	41	155	36	109	19	138	81
3-Methyl-2-butenol	ns	***	32	14	62	10	79	54	291	42	33	16	59	9	45	13	76	25
3-Methyl-1-pentanol	*	**	35	19	80	14	49	29	140	24	28	11	46	12	33	15	60	20
Hexanol	ns	***	3798	624	5120	2774	5853	2713	13,646	3817	4030	1154	5540	1908	4969	763	6283	4110
t-3-Hexenol	**	***	26	6	45	16	40	23	109	23	21	6	32	11	27	8	39	17
c-3-Hexenol	***	ns	173	71	312	69	242	122	505	88	101	42	196	61	142	65	255	68
Benzylic	**	***	200	112	414	24	398	179	863	195	141	103	304	<QL	229	86	375	93
Ethyl esters																		
Propionate	ns	***	209	142	550	57	1898	1062	4567	518	190	130	467	71	1424	1556	5529	130
Butyrate	ns	***	116	49	279	57	168	52	277	97	115	37	178	56	160	44	243	102
2-Methylbutyrate	ns	***	22	8	45	6	47	16	85	24	35	11	47	16	30	19	63	12
Hexanoate	ns	**	147	58	309	51	195	83	398	98	143	54	244	50	177	65	266	81
Octanoate	*	ns	207	88	414	80	228	116	479	92	158	31	216	115	177	75	315	86
3-Hydroxybutyrate	ns	ns	126	100	403	28	180	87	414	81	148	56	256	55	148	89	349	65
4-Hydroxybutyrate[b]	***	ns	799	277	1424	419	855	454	2381	414	522	208	1001	200	594	282	1116	266
Acetates																		
Propyl	ns	***	28	11	57	16	142	69	341	62	31	6	42	23	51	20	101	31
Butyl	*	ns	52	19	83	5	46	5	53	37	48	18	89	33	43	3	47	38
Isoamyl	***	ns	619	447	1944	82	470	253	1159	150	183	119	468	45	243	125	480	139
2-Phenylethyl	***	ns	282	367	1584	<QL	104	71	285	27	42	26	95	<QL	48	44	130	<QL
Miscellaneous compounds																		
3-Ethoxy-1-propanol	**	***	218	192	735	<QL	372	258	1128	119	69	46	179	<QL	285	282	809	<QL
γ-Butyrolactone	**	***	512	200	957	188	2008	776	4362	1039	1137	596	2299	398	2641	1771	7077	1058
Methionol	ns	**	448	219	1066	220	639	296	1599	289	488	123	669	247	622	282	1221	312

Mat: maturation; SD: standard deviation; Max: maximum; Min: minimum; nd: not detected; QL: quantification limit; ns: not significant.

* Significant at $p < 0.10$.

** Significant at $p < 0.05$.

*** Significant at $p < 0.01$.

[a] Sum of amyl and isoamyl alcohols.

[b] Quantified as ethyl 3-hydroxybutyrate.

the predominance of *Saccharomyces bayanus* species during the tumultuous fermentation step (Querol, Fernández-Espinar, del Olmo, & Barrio, 2003; Suárez Valles et al., 2005). The spontaneous fermentations characterized in Asturias showed that the genus *Hanseniaspora* is predominant at the beginning of the process. The activity of non-*Saccharomyces* yeast strains is associated with the production of acetate esters, such as isoamyl and 2-phenylethyl acetates (Xu, Zhao, & Wang, 2006), which contents were significantly higher in Asturian ciders. These yeasts are subsequently substituted by yeasts of the genus *Saccharomyces bayanus*, and *Saccharomyces cerevisiae* at the end (Suárez Valles et al., 2007).

The maturation of ciders significantly increased the levels of acetaldehyde, methanol, 2-butanol, 1-propanol, and ethyl lactate, and within minor volatiles, 4-ethylphenol, 4-ethylcatechol, four alcohols (3-methyl-3-butenol, 1-pentanol, 3-methyl-2-butenol and hexanol), ethyl esters (propionate, butyrate, 2-methylbutyrate and hexanoate), propyl acetate and methionol, and decreased the concentrations of decanoic acid in the Asturian samples.

These increases could be related to the capacity of lactic acid bacteria to synthesize ethyl esters and other volatile compounds such as fatty acids, volatile phenols, alcohols and γ-butyrolactone (Beech & Carr, 1977; Sumby, Grbin, & Jiranek, 2010; Ugliano & Moio, 2005). Moreover, the influence of the contact of ciders with lees and the ability of *Lactobacillus collinoides* species to produce 4-ethylcatechol – among other volatile phenols- from hydroxycinnamic acids have been ascertained (Antón-Díaz et al., 2016; Buron et al., 2011b). Other components were influenced by both origin and maturation. In general, it was observed that Asturian matured ciders exhibited the highest levels, except those for 4-vinylguaiacol, which decreased or disappeared, as in the Basque older samples (Table 3). The decrease of 4-vinylguaiacol in the matured samples could suggest the implication of a 4-vinylreductase activity of some lactic acid bacteria (Couto, Campos, Figueiredo, & Hoggs, 2006).

3.3. Olfactometric profiles

The olfactometric profiles of ciders are presented in Table 4. There were taken into account 57 olfactometric peaks, twenty of them being present in all of the samples. Those odorants included major compounds, such as amyl alcohols (v8) and 2-phenylethanol (v47), minor components, like 4-ethylguaiacol (v52) and 4-ethylphenol (v64), and not quantified volatiles, such as 1-octen-3-one (v12).

The olfactometric profiles were, in general terms, consistent with the observed quantitative trends, although important differences must be considered. On the one hand, relevant components for cider maturation like 4-ethylcatechol was not perceived by olfactometry, whereas others such as γ-butyrolactone or hexanol had less influence; on the other, there were relevant contributions of minor and not quantified but potent odorants, such as 1-octen-3-one, cresols, and eugenol, which may be present at the level of traces. This capacity makes the Gas Chromatography-Olfactometry method a valuable complementary support to the quantitative analytical tool that has shown its ability to successfully discriminate ciders by their place of origin and maturity stage.

The origin of the ciders significantly influenced the perception of up to thirteen odorants, 2-methylbutanoic (v36), ethyl 4-hydroxybutanoic (v42), an two unknown odorants (v51 and v55) being highly significant. In general, Asturian ciders had the highest mean intensity values. Likewise, maturity of ciders gave significant rises of up to 10 odorants. Among these, it is worth to mention the increases shown by 1-octen-3-one (v12) and acids such as isobutyric (v30), hexanoic (v44) and octanoic (v54).

3.4. Typification of ciders

Discriminant-PLS multivariate analyses were performed to classify the ciders according to their geographical origin and maturation. It can be said that the volatile composition did provide satisfactory results only for modeling maturation of ciders, whereas the use of the olfactometric profiles as predictor variables allowed the ciders to be classified by both origin and maturation.

The mathematical approach obtained to discriminate cider maturation from volatile composition explained 66% of the variance, with a standard prediction error (SPE) of 0.29. As shown in Fig. 1A, young ciders are placed on the positive side of the first principal component axis, and matured ciders on the negative side. This model identified two outliers, referred to as A21 (Asturian young cider), and B10 (Basque matured cider), and gave the misclassification of the ciders referred to as A2 (Asturian matured), A32, B14 and B20 (young samples). The content of 4-ethylcatechol was strongly associated with matured ciders, followed by ethyl propionate, γ-butyrolactone, hexanol, octanoic and hexanoic acids, and 4-ethylphenol, while 4-vinylguaiacol was associated with the young ones (Fig. 2A). Sample referred to as A21 presented the highest content of 4-ethylcatechol of the cider population analyzed (9711 μg/L), whereas B10 had the highest level of γ-butyrolactone (7077 μg/L) and a moderated concentration of 4-ethylcatechol (4201 μg/L). The results obtained in this study showed that the relationship between 4-vinylguaiacol and 4-ethylcatechol was higher among the young samples, excepting those referred to as A32, B14 and B20 (young ciders), wrongly predicted as matured ciders (data not shown).

Taking into account the olfactometric profiles, the discriminant model obtained for cider maturation did explain 90% of the variance (SPE, 0.16). Figs. 1B and 2B respectively show the projections of ciders and variables onto the plane formed by the first two principal components. As seen in Fig. 1B, the first axis separated ciders according to their maturation stage, young ciders being placed on the right side, and the matured ones on the left. The most correlated variables were components such as v12 (1-octen-3-one) and v13 (ni, LRI 1299), together with acids such as propanoic (v29), isobutanoic (v30), and octanoic (v54), and volatile phenols such as *m*-cresol (v58), and eugenol (v63), as shown in Fig. 2B.

Finally, Figs. 1C and 2C respectively display the projection of ciders and predictive variables onto the plane formed by the first two principal components. This model explained 77% of the variance attributed to cider origin (SPE, 0.23). As seen in Fig. 1C, the first axis allowed grouping of samples according to this factor, most of the Asturian ciders being placed on the left side, while the PC2 also discriminated the samples on the basis of their maturation stage (matured ciders, on the positive side of the second axis, young ones, on the negative side). The most correlated variables with the PC1 were a sweet odorant (v55) and *p*-cresol (v57). The PC2 was correlated with propyl acetate (v2), 1-octen-3-one (v12), associated to the Asturian matured samples, and the toasty odorant referred to as v13 strongly linked to the Basque young ciders (Fig. 2C).

Odorant components like 1-octen-3-one have been described in apple pomace (Rodríguez Madrera, Pando Bedriñana, & Suárez Valles, 2015) and as a potent odorant of ciders (Antón et al., 2014; Villière, Arvisenet, Lethuaut, Prost, & Sérot, 2012). Likewise, Williams, May, and Tucknott (1978) reported its presence in ciders and that the mushroom odour had been commonly noted in ciders made from bittersweet apples. Moreover, this cetone, ubiquitous in foods and beverages, is also a common odorant of the olfactometric profiles of normal wines (Culleré, Cacho, & Ferreira, 2006). The existence of increasing amounts of eugenol has been reported in distillates made from ciders with increased degrees of maturation (Rodríguez Madrera, Picinelli Lobo, & Mangas Alonso, 2010).

Table 4
Olfactometric profiles of Spanish natural still ciders: olfactive description, identity, mean intensities (mean, maximum and minimum values).

							Asturias						Basque Country					
							Young			Matured			Young			Matured		
LRI_{WAX}	LRI_{DB5}	Description	Identity	RefV	Origin	Mat	M	Max	Min	M	Max	Min	M	Max	Min	M	Max	Min
905	719	Fruity	Ethyl propionate	v1	*	ns	5	23	0	10	28	0	23	48	10	9	42	0
931	721	Fruity	Propyl acetate	v2	***	**	31	45	13	32	53	14	11	18	4	31	45	7
1057	804	Fruity	Ethyl butyrate	v4	ns	ns	22	43	4	19	38	10	20	38	7	22	32	13
1074	805	Fruity, apple-like	Ethyl 2-methylbutyrate	v5	ns	ns	40	55	25	43	62	27	43	52	32	41	52	25
1144	879	Fruity, banana	Isoamyl acetate	v7	**	ns	15	38	0	13	27	0	4	13	0	7	17	0
1217	753	Like fusel alcohols	Amyl alcohols	v8	ns	ns	46	60	30	42	60	30	38	50	22	44	52	33
1240	1183	Ripen fruit	Ethyl hexanoate	v10	ns	ns	27	40	18	22	42	0	23	35	10	25	43	13
1294	983	Mushroom	1-Octen-3-one	v12	ns	***	20	57	4	54	67	33	5	8	3	51	58	42
1299		Toasty, smoky	ni	v13	ns	***	40	57	4	9	27	4	53	58	43	8	17	4
1364	889	Herbaceous, floral	Hexanol	v15	ns	ns	8	20	0	9	42	0	4	17	0	6	22	0
1383	887	Floral, geranium	*t*-3-Hexenol	v16	ns	***	26	46	4	37	68	17	23	38	10	34	57	22
1404	867	Floral	*c*-3-Hexenol	v17	ns	***	8	23	0	14	33	0	5	17	0	21	50	7
1419		Sweet, spicy	ni	v19	**	ns	1	3	0	1	10	0	0	0	0	0	0	0
1440	1190	Fruity, resin	Ethyl octanoate	v21	ns	**	7	20	0	6	30	0	7	20	0	2	13	0
1448		Vinegar-like, fusel alcohols	Acetic acid	v22	*	***	39	56	15	49	60	37	34	44	20	45	57	33
1466	908	Dust, rancid, cooked vegetables	Methional	v23	**	***	9	35	0	22	50	3	5	20	0	9	23	0
1477		Spicy, cooked vegetables	ni	v24	***	***	12	40	0	26	40	5	0	0	0	11	17	0
1494	957	Fruity, floral	Ethyl 3-hydroxybutyrate	v27	ns	***	6	26	0	23	47	0	6	17	0	16	42	0
1508		Fatty, fruity	ni	v28	***	***	18	43	3	0	0	0	0	0	0	0	0	0
1519	856	Fatty, stable	Propanoic acid	v29	**	***	3	30	0	26	45	7	13	20	0	29	33	23
1529	909	Fatty, heavy	*iso*-Butyric acid	v30	ns	***	6	32	0	32	62	7	7	18	0	26	53	0
1554	1096	Fatty, resin	1-Octanol	v32	ns	ns	8	28	0	11	27	0	11	20	3	7	17	0
1567	966	Lactic, blue cheese	Butyric acid	v33	**	***	10	24	0	0	0	0	11	20	0	5	13	0
1635	954	Rancid, varnish	γ-Butyrolactone	v34	ns	*	55	67	44	56	68	43	51	64	40	54	65	20
1681		Cheese, rancid	2-Methylbutyric acid	v36	***	ns	60	72	42	60	72	47	53	64	43	51	58	40
1735	1013	Cabbage	Methionol	v38	ns	***	50	60	35	39	52	15	47	54	32	39	47	31
1768		Broth-like	ni	v40	**	**	4	25	0	0	0	0	0	0	0	0	0	0
1775		Floral, coconut	ni	v41	*	**	5	18	0	0	0	0	0	0	0	4	27	0
1801		Sweet, fruity, floral	Ethyl 4-hydroxybutyrate[a]	v42	***	ns	14	45	0	12	37	0	2	3	0	2	13	0
1837	1267	Stewed fruit, floral	2-Phenylethyl acetate	v43	ns	ns	53	65	35	51	63	40	54	62	38	56	63	50
1860	1174	Fatty, stable	Hexanoic acid	v44	ns	***	48	65	36	57	73	17	44	57	27	58	68	43
1880	1118	Sweet, smoky, spicy	Guaiacol	v45	*	ns	14	40	0	17	35	0	12	28	0	9	17	0
1903	1071	Fruity, sweet	Benzyl alcohol	v46	ns	ns	25	52	4	25	40	12	17	28	0	26	47	10
1929	1175	Roses	2-Phenylethanol	v47	ns	ns	61	78	36	64	77	55	58	64	47	61	68	50
1986		Floral, spicy	ni	v49	**	**	5	24	0	11	44	0	3	20	0	3	13	0
2001	1088	Smoky	*o*-Cresol	v50	ns	ns	14	40	0	9	28	0	9	28	0	8	13	3
2023		Fatty, spicy	ni	v51	***	ns	7	33	0	7	22	0	0	0	0	0	0	0
2047	1294	Sweet spicy	4-Ethylguaiacol	v52	ns	**	62	77	37	67	80	38	57	70	50	69	82	62
2078	1358	Fatty, sweat	Octanoic acid	v54	ns	***	15	52	3	41	63	7	16	30	7	46	65	10
2079		Sweet, spicy	ni	v55	***	ns	44	68	10	26	43	7	0	0	0	14	20	10
2100	1123	Leather, stable	*p*-Cresol	v57	*	ns	15	62	0	35	53	13	51	58	40	14	20	10
2109	1099	Leather, stable	*m*-Cresol	v58	ns	***	27	47	10	8	33	0	29	43	13	3	13	0
2143		Sweet, spicy	ni	v59	ns	**	0	0	0	7	30	0	8	32	0	0	0	0
2169		Floral, sweet	ni	v61	**	***	24	40	0	12	35	0	23	40	0	0	0	0
2171	1476	Spicy, sweet	γ-Decalactone	v62	**	ns	23	45	0	35	60	0	25	48	0	12	20	7
2183	1385	Spicy, clove	Eugenol	v63	ns	***	2	27	0	24	72	0	0	0	0	14	20	7
2194	1165	Leather, stable	4-Ethylphenol	v64	ns	ns	54	75	37	56	73	33	54	65	45	57	67	45
2210	1095	Smoky, spicy, curry	Sotolon[a]	V66	ns	ns	61	77	53	68	77	57	62	68	55	66	77	55
2244		Spicy, smoky	ni	v67	ns	**	13	22	0	11	23	0	18	30	7	8	13	3
2246	1279	Spicy	4-Vinylguaiacol	v68	**	***	35	53	17	20	48	4	28	45	10	15	20	4
2252		Spicy, sweet	ni	v69	***	***	0	0	0	12	37	0	0	0	0	0	0	0
2261	1913	Resin, grass-like	Ethyl hexadecanoate	v70	*	ns	11	34	0	20	60	0	12	50	0	7	13	0
2285	1451	Fatty, sweat	Decanoic acid	v71	ns	ns	56	68	24	50	70	7	54	65	32	53	68	30
2297		Stable, phenolic	ni	v72	**	ns	21	44	0	22	58	0	19	47	0	6	10	0
2333		Stable, phenolic	ni	v75	ns	***	0	0	0	9	20	0	0	0	0	5	13	0
2358		Stable, floral	ni	v76	**	ns	6	30	0	4	27	0	0	0	0	3	10	0
2360	1496	Smoky, spicy	Isoeugenol	v77	**	ns	3	20	0	3	13	0	7	20	0	5	13	0

LRI: Linear Retention Indexes on column DB-WAX or DB5; RefV: reference for variables; M: mean; Max: maximum; Min: minimum; ni: not identified.

[a] Temptatively identified.

[*] Significant at $p < 0.10$.

[**] Significant at $p < 0.05$.

[***] Significant at $p < 0.01$.

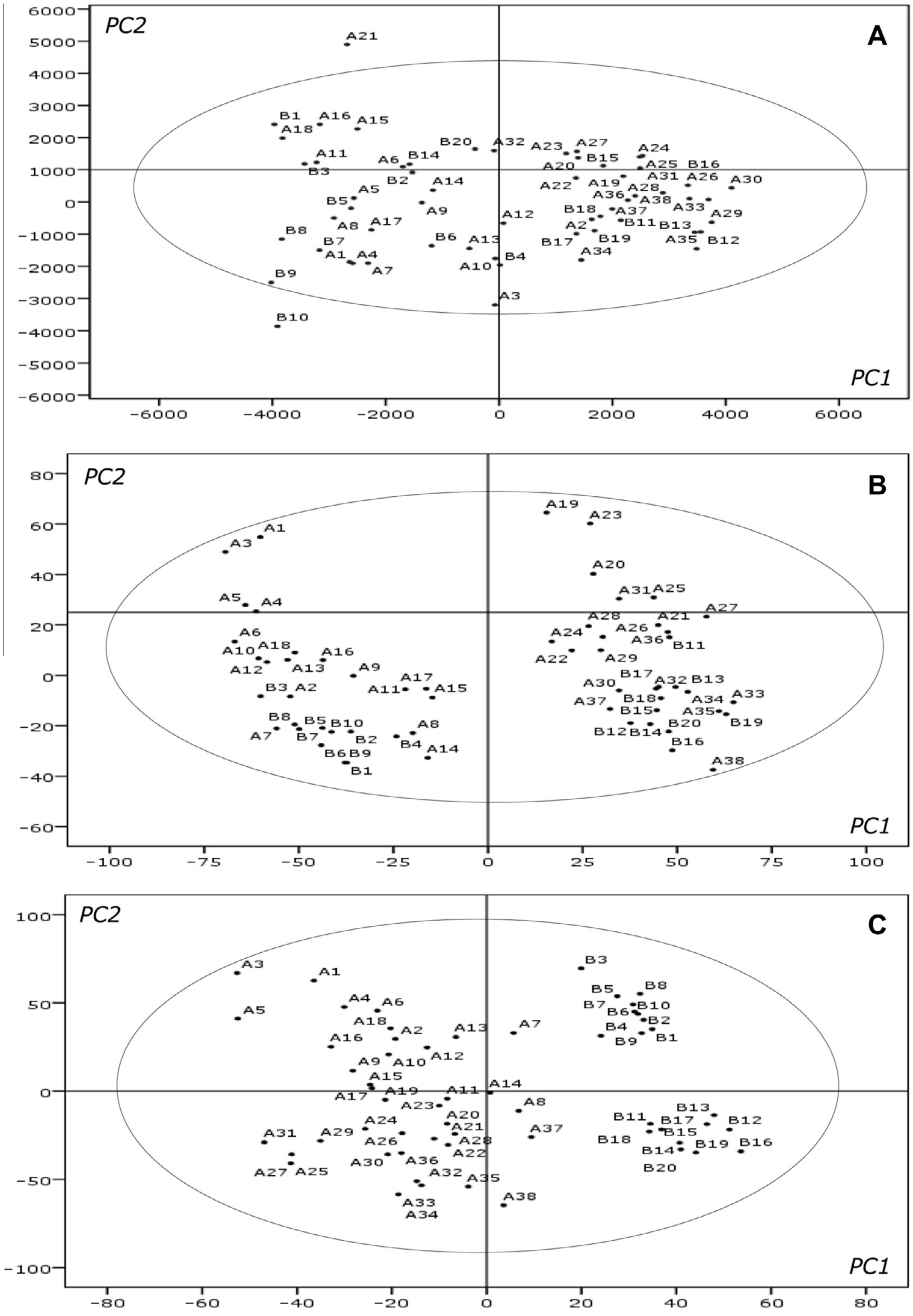
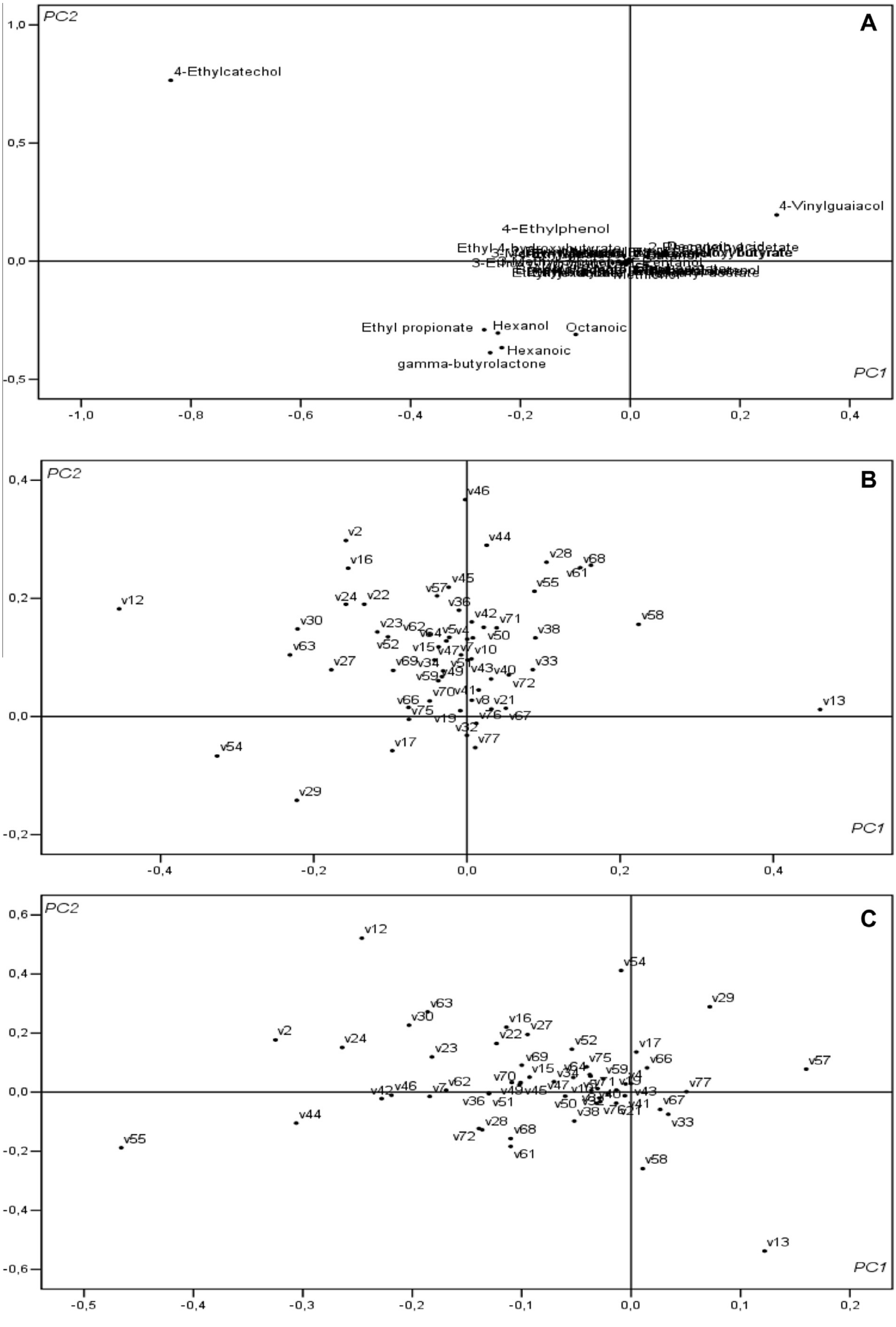


Fig. 1. Projection of cider scores on the factorial plane formed by the first two principal components together with the T^2-Hotelling distribution ellipse at a 95% confidence level. A: Asturian ciders; B: Basque ciders; matured ciders: A1–A18 and B1–B10; young ciders: A19–A38 and B11–B20. (A) Discriminant-PLS model for maturation stage of ciders, taking quantitative data as predictive variables. (B) Discriminant-PLS model for maturation stage, taking the olfactometric profiles as predictive variables. (C) Discriminant-PLS model for origin of ciders, taking the olfactometric profiles as predictive variables.

Fig. 2. Projection of variable loadings onto the plane formed by the first two principal components. Cider codes as in Fig. 1. For variable identification, see Table 4. (A) Projection of volatile composition from discriminant-PLS model for maturation stage of ciders. (B) Projection of the olfactometric profiles from discriminant-PLS model for maturation stage of ciders. (C) Projection of the olfactometric profiles from discriminant-PLS model for origin of ciders.


A
PC2
PC1
4-Ethylcatechol
4-Vinylguaiacol
4-Ethylphenol
Ethyl 4-hydroxybutyrate
Decanoic acid
Ethyl propionate
Hexanol
Octanoic
Hexanoic
gamma-butyrolactone
1,0
0,5
0,0
-0,5
-1,0
-0,8
-0,6
-0,4
-0,2
0,0
0,2
0,4
B
PC2
PC1
v46
v2
v44
v28
v68
v16
v61
v45
v55
v57
v12
v24
v22
v36
v42
v58
v71
v30
v23
v62
v5
v4
v38
v64
v50
v63
v52
v15
v47
v7
v10
v27
v69
v34
v51
v33
v43
v40
v59
v49
v72
v70
v41
v13
v66
v8
v21
v75
v76
v67
v19
v32
v77
v17
v54
v29
0,4
0,2
0,0
-0,2
-0,4
-0,2
0,0
0,2
0,4
C
PC2
PC1
v12
v54
v63
v29
v30
v16
v27
v2
v22
v52
v17
v24
v23
v66
v69
v57
v75
v15
v64
v59
v34
v4
v70
v77
v47
v71
v62
v19
v42
v46
v7
v49
v45
v40
v43
v67
v36
v51
v50
v41
v38
v76
v21
v33
v44
v28
v68
v72
v61
v55
v58
v13
0,6
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,5
-0,4
-0,3
-0,2
-0,1
0,0
0,1
0,2

4. Conclusions

Asturian and Basque ciders were clearly differentiated by their volatile composition and olfactometric profiles. In general, higher volatile contents were observed for the Asturian and matured ciders. The content of 4-ethylcatechol, was highly associated with the older samples whereas 4-vinylguaiacol was linked with young ciders. Although more research is needed to determine the influence of the making process on the aromatic profile of this product, the use of the olfactometric profiles gave promising results to classify ciders by origin or maturation; a non identified-sweet odorant, *p*-cresol, propyl acetate, and 1-octen-3-one were correlated to the origin of ciders, whereas this cetone, together with acids such as octanoic, propanoic and isobutanoic, eugenol, *m*-cresol, and one unknown spicy-vegetal odorant were correlated to the maturation stage.

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