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Chemical, olfactometric and sensory description of single-variety cider apple juices obtained by cryo-extraction

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ABSTRACT

A set of ten single-apple juices obtained by cryo-extraction has been analysed for chemical and sensory profiles to evaluate their potential for the production of ice juices. Criteria for the selection of apple varieties were based on the equilibrium between sugars and acids and aromatic complexity. Verdialona, Durona de Tresali and Perico presented the highest ratios between total sugars and total acidity, while Raxao and Regona had the lowest. The results for olfactometry show that Xuanina, Limón Montés and Perico had the most complex olfactometric profiles and de la Riega, the simplest. Sensory analyses revealed that the juice from Durona de Tresali was the most appreciated, followed by those from de la Riega, Solarina and Blanquina cultivars. Verdialona was described by its sweet taste whereas the Regona cultivar was mainly defined by its tannin/bitter flavour.

1. Introduction

Nowadays, the Asturian cider industry is involved in a product diversification process which includes the development of beverages similar to so-called ice ciders. This cider, inspired by ice wines, was first made in Quebec in the early 90s, and had soon attracted great interest worldwide, reaching significant export markets in Europe and Asia (Leger, 2010, p. 42). Ice cider is a balanced sweet-acid tasting beverage with an intense and complex aromatic profile, described as apple, exotic fruits, vegetal, nutty or mushrom-like (Clément, Panneton, Bastien, & Fernandez, 2017; Fernandez, 2017).

The distinctive characteristics of ice cider are deeply associated to its special making procedure. The raw starting material is an apple juice concentrated by freezing which contains high concentrations of sugars and malic acid to balance the final product. There are two methods to obtain this enriched juice. Cryo-concentration or "cold-freeze concentration", in which the fruit is pressed and the resulting juice is frozen; the second method, called cryo-extraction, consists of pressing frozen apples. The first technique is the most frequently used although some producers claim that the ice ciders obtained by cryo-extraction are often more complex and aromatic (Kirkey & Braden, 2014).

The fermentation process following the enrichement of the apple juice is developed through inoculation with yeasts, which ends while there is a still a residual sugar content (Nurgel, Pickering, & Inglis, 2004). In fact, due to the high concentration of sugars typically present in these types of juices, yeasts are subjected to large hyperosmotic stress and therefore, the selection of the most suitable strains is necessary to avoid sluggish fermentations and to obtain the desired alcoholic degree. Strains of *Saccharomyces bayanus* have recently been evaluated for the production of ice ciders (Pando Bedriñana, Mangas Alonso, & Suárez Valles, 2017).

Regarding ciders, the selection of the proper apple varieties may be one important factor influencing the creation of well differentiated or unique ciders. The objective of selecting the best cider cultivars for ice cider making has not been evaluated. While four dessert apple varieties are most often used in Canada for cider-making (McIntosh, Cortland, Empire and Spartan), Asturias possesses a huge collection of cider apple cultivars from which to choose. Today, 516 cider varieties are included in SERIDA's Bank of Germoplasm. Several research activities have been undertaken for the molecular and agronomical characterization of those

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cultivars, the selection and the improvement of the most suitable from the technological point of view. Some of the resulting selected cider apple varieties (22 cultivars) have been included in the regulation of the Protected Designation of Origin “Cider from Asturias” (EC 2154/2005).

In this paper, the cryo-extraction technique has been chosen to obtain the enriched juices. Ten cider apple varieties, selected among those authorised by the above mentioned regulation and widely cultivated in Asturias have been evaluated using chemical, olfactometric and sensory characteristics to assess their potential for the making of ice juices.

2. Materials and methods

2.1. Apple juices

Ten cider apple varieties (Verdialona, Solarina, Blanquina, de la Riega, Perico, Durona de Tresali, Raxao, Limón Montés, Xuanina and Regona) belonging to the Protected Designation of Origin “Cider from Asturias” were collected from an apple grove situated in Villaviciosa (Asturias) at their optimal stage of maturation as determined by the iodine starch test.

Around 20–30 kg of each variety were frozen and kept at −20 °C until processing in a local cellar. At that moment, the apples were pressed by means of a hydro-pneumatic press (200 kPa) when they reached a temperature of −9 °C (measured inside the apple). The juice squeezed from each variety was taken so as to reach a final soluble solid content as close as possible to 30 ºBrix. The extraction process was performed at cellar temperature (8–10 °C); it took between 30 and 40 min, depending on the cultivar. The juices were not clarified, and were subsequently stored at 4 °C until chemical and sensory analyses were performed.

2.2. Reagents and standards

The volatile and phenolic standards were supplied by Sigma (St. Louis, MO), Aldrich (Gillingham, U.K.), Fluka (Buchs, Switzerland) and Extrasynthèse (Genay, France). Dichlorometane, ammonium sulphate, anhydrous sodium sulphate, methanol and acetic acid were from Panreac (Barcelona, Spain), and pentane from VWR (Darmstad, Germany). All the reagents were of chromatographic quality.

2.3. Oenological parameters

The analyses for density (g/mL), pH, total acidity (g malic acid/L), and total polyphenol (Folin-Ciocalteu index, g tannic acid/L) were done according to the European Union Official Methods of Analysis (1998). Sugars and polyalcohols were determined by HPLC using a cation exchange column (SugarPak I 300 × 6.5 mm, from Waters, Milford, MA, USA) and a refraction index detector. Organic acids were analysed by RP-HPLC on a Spherisorb ODS2 column (250 × 4.6 mm; 3 µm from Waters, Milford, MA, USA) eluting in isocratic mode (buffer at pH = 2.40), and diode array detection at three wavelenghts (malic acid, 206 nm; shikimic acid, 212 nm and ascorbic acid, 245 nm), as reported elsewhere (Picinelli et al., 2000).

2.4. Polyphenol analysis

Phenolic profiles were determined by means of a Waters system equipped with a 717 automatic injector, a column oven set at 30 °C, two M510 model pumps, a 2996 diode array detector, a 2475 fluorescence detector and a Millenium Empower data module. Polyphenols were separated on a Nucleosil 120 C_{18} column (250 × 4.1 mm; 3 µm from Macherey-Nagel, Panreac, Barcelona, Spain) and gradient elution with acetic acid (20 mL/L solvent A) and methanol (solvent B), as described elsewhere (Suárez, Palacios, Fraga, & Rodríguez, 2005). Detection was performed at 280 nm for dihydrochalcones, at 313 nm for hydroxycinnamic acids and their derivatives, and at 355 nm for flavonols. Flavanols and procyanidins were analized by fluorescence (λ_{ABS} = 280 nm; λ_{EM} = 317 nm). Quantitation was done by the external standard method. For those analytes where no standard was available, standards of their same family were used; thus, procyanidins were quantified as (+)-catechin, flavonols as quercitrin, dihydrochalcones as ploridzin, and phenolic acids as chlorogenic acid or *p*-coumaric acid.

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 extracts of the different juices were prepared by one step liquid-liquid extraction with a mixture of pentane:dichloromethane (2/1, v/v). The odorants were identified by comparing their odour characteristics and retention indexes with those of the reference standards, and their corresponding spectra by GC-MS, as reported previously (Antón, Suárez Valles, García Hevia, & Picinelli Lobo, 2014). A panel of 6 people participated in the sniffings of the extracts; each judge assesssed the samples in one session (35 min). The data obtained were a combination of intensity (I, %) and frequency of detection (F, %), called modified frequency:

$$MF(\%) = \sqrt{F(\%)xI(\%)}$$

Those peaks not reaching a GC-O score of 30% MF in at least one of the samples were not taken into account for subsequent analyses.

2.6. Sensory analysis

Eight judges (aged from 30 to 55) belonging to the Sensory Committee of the Protected Designation of Origin “Cider from Asturias” were scheduled to assess the samples in three sessions. In the first one, only two juices were evaluated to become familiar with these kinds of samples. The panel selected by consensus six attributes to describe aroma and mouthfeel: Applesauce/Ripe apple, Green apple, Ripe fruit, Green fruit, Grassy and Tannin/bitter. These data were evaluated on the basis of their citation frequencies. In addition, two quantitative scales were established: a 3-point scale (defined as follows: 3, high intensity or persistence; 2, moderate, and 1, low) to measure the intensities for odour, sweetness, acidity, and after-taste persistence; and a 5-point scale (defined as follows: 5, excellent; 4, good; 3, fair/correct, 2, not fair, and 1, defective), to score the assessments for qualities for odour and taste, and overall impression.

This procedure was used further to assess the juice samples in two sessions. The ice juices were randomly presented in normalised glasses (ISO 3591:1977), and evaluated at 10–12 °C.

2.7. Statistical analyses

A non-parametric Kruskall-Wallis test was performed to evaluate the effect of apple varieties on the sensory and olfactometric profiles. A categorical principal component analysis (CAT-PCA) was performed to ascertain relationships between sensory attributes, and olfactometric profiles and chemical parameters. Data matrix consisted of 10 objects (apple juices) and 52 variables, both continuous (composition data and citation frequencies, previously normalised) and categorical (quality assessments, intensities of odour, sweetness and acidity, and after-taste persistence). All the analyses were done by using the statistical package SPSS v.12.0 for Windows (SPSS Inc., Chicago, Ill., U.S.A.).

Table 1
Chemical and technological description of the ice apple juices obtained by cryo-extration.

Technological group	PSD	Verdialona	Solarina	Blanquina	De la Riega	Perico	Durona de Tresali	Raxao	Limón Montés	Xuanina	Regona
		Sweet	Semi-acid	Acid	Semi-acid	Semi-acid	Acid	Acid	Acid	Acid	Acid-bitter
Extraction yields (%)		21	18	20	8	11	18	14	26	13	12
Density (g/mL)	1.2E-6	1.1173	1.1137	1.1040	1.1035	1.1090	1.1084	1.1083	1.1114	1.1112	1.0940
Total Soluble solids (ºBrix)	0.03	28.0	27.2	25.1	25.0	26.2	26.0	26.0	26.7	26.7	22.9
pH	1.0E-3	3.46	3.20	3.04	3.14	3.25	3.37	3.12	3.25	3.11	3.07
Total acidity (g malic acid/L)	0.02	11.01	17.12	20.08	17.49	13.46	12.92	25.54	16.67	22.06	20.50
Total phenols (g tannic acid/L)	3.2E-3	1.9	3.2	2.1	3.4	2.6	2.4	2.3	2.3	3.3	3.5
Sucrose (g/L)	0.16	46.8	37.4	39.6	37.7	52.8	43.9	24.9	79.3	39.8	30.5
Glucose (g/L)	0.10	44.9	40.5	32.1	42.2	40.1	32.8	38.7	33.5	28.9	42.8
Fructose (g/L)	0.31	131.0	130.7	115.3	121.2	115.5	123.2	120.6	92.4	137.8	93.5
Sorbitol (g/L)	0.06	20.7	18.7	13.1	7.8	14.7	19.7	16.2	13.9	14.2	13.8
Malic acid (g/L)	0.17	11.5	18.1	19.1	17.4	13.7	13.7	24.8	17.3	21.4	19.6
Shikimic acid (mg/L)	0.96	33	51	18	29	32	18	34	32	28	66
Ascorbic acid (mg/L)	0.65	85	173	18	40	41	nd	nd	nd	17	6
Total sugars/total acidity	0.15	20.2	12.2	9.3	11.5	15.5	15.5	7.2	12.3	9.4	8.1

Values shown are mean of two determinations; PSD: pooled standard deviation; nd: not detected.

3. Results and discussion

The apple varieties taken for study are widely used for the making of Asturian cider. This beverage is characterised by a strong flavour, with a good balance between sharpness and bitterness so that, the majority of the cider cultivars belong to technological groups with acidic characters (Table 1).

3.1. Chemical composition

The criteria for the assessments of the apple cultivars for the making of ice juices were based on their oenological characteristics, mainly sugar and acid contents, and aromatic complexity.

Total sugar contents were comprised between 166.8 g/L for Regona cultivar and 222.6 g/L for Verdialona. Fructose was the major component, followed by glucose and sucrose (Table 1). Total soluble solid contents (ºBrix) ranged between 22.9 for Regona *cv*. and 28.0 for Verdialona, these values being almost two-fold that of the conventionally pressed musts. The content of total soluble solids is high in the first pressing fractions, decreasing as processing time increases due to the thawing of the ice crystals inside the apple tissues (Carbonell-Capella et al., 2016). This fact greatly influences the yield of the technological process the objective of which is to obtain a final value for total soluble solids as close as possible to 30 ºBrix in the final juice. In this set of cider apple varieties, the cryo-extraction method used to obtain the apple musts gave a wide range of yields (%). The de la Riega cultivar had the lowest value (8%) whereas Limón Montés, Verdialona and Blanquina had the highest (Table 1).

Contents of malic acid varied between 11.5 and 24.8 g/L. This acid could be relevant to the taste and mouthfeel of the apple juices because of its sour and astringent properties (CoSeteng, McLellan, & Downing, 1989; Corrigan Thomas & Lawless, 1995). The relationship between total sugars and total acidity is a key parameter in the making of this type of product, with the aim of obtaining an equilibrated flavour. This value ranged between 7.2 for the most acidic variety (Raxao) and 20.2 for the sweetest one (Verdialona).

Total phenol contents in the cryo-extracted juices were included between 1.9 and 3.5 g tannic acid/L. Compared with conventionally pressed juices, and depending on the year of harvest and their maturity stage, normal values for all these cultivars ranged between 0.8 and 1.9 g tannic acid/L (Mangas, Rodríguez, Suárez, Picinelli, & Dapena, 1999).

3.2. Phenolic profiles

Twenty-four components, belonging to the four main classes of phenolics usually reported in apples were quantified (Table 2). Hydroxycinnamic acids represented between 50% and 70% of the phenolic composition, chlorogenic acid being the major compound in all the juices. The unknown component referred to as AC2, which had the same spectral characteristics as chlorogenic acid, was the second most important phenolic acid except in the Regona cultivar, in which the *p*-coumaroylquinic acid content reached 140 mg/L. Other acidic compounds with the same spectrum as *p*-coumaric acid (AC1 and AC3) were also quantified. The de la Riega and Xuanina cultivars had the highest contents of phenolic acids, while Verdialona *cv.* had the lowest. Hydroxycinnamic acids are the most extractable phenolic group (Malec et al., 2014), which could partially explain their high contents in the cryo-extracted juices. Flavan-3-ols and procyanidins, followed by dihydrochalcones were also abundant components of the phenolic composition of these apple juices. The first family ranged between 16% and 36% of the low molecular weight phenolic composition, with Solarina, Regona and de la Riega *cv*. having the highest contents. Flavan-3-ols and procyanidins are influenced by the extraction conditions, as they are sensitive to both oxidation and the temperature at which pressing is performed. It has been reported that the extraction of procyanidins from fruit to juice decreases when the pressing process is done at low temperatures (Renard et al., 2011). The group of dihydrochalcones ranged between 7% and 27% of the phenolics analysed, varieties Regona and Perico *cv.* showing the highest percentages. These components are retained in the pomace, their extractability varying among apple cultivars and with the processing conditions (Le Bourvellec, Le Quéré, & Renard, 2007; Renard et al., 2011). Finally, the group of flavonols, accounted for only 1–3% of the phenolic profile. Six quercetin glycosides were found with hyperin, avicularin and quercitrin being the major ones. These components are mainly located in the apple peel and therefore, their content in juices obtained by pressing is very low as they remain attached to the apple pomace (Alonso-Salces et al., 2004; Diñeiro García, Suárez Valles, & Picinelli Lobo, 2009).

3.3. Olfactometric profiles

The results for olfactometric analyses of the apple juices are shown in Table 3. Thirty-two (32) odorants were observed, of which 19 allowed differentiation among apple varieties. Only six odorants were present in all samples (v7, v15, v16, v24, v29 and v31). Xuanina (J9), Limón Montés (J8) and Perico (J5) had the most complex olfactometric profiles, whereas de la Riega (J4) had the simplest, as 21 odorants were absent in this juice. Esters and alcohols are the most abundant volatile compounds present in apples, their concentrations being related to varieties, maturity stage and the storage conditions of apples (Dixon & Hewett, 2000; Mehinagic, Royer, Symoneaux, Jourjon, & Prost, 2006). Other odorants such as 2-phenylethanol, 2-methylbutyric acid, 4-

Table 2
Phenolic profiles (mg/L) and pooled standard deviation of the ice apple juices.

	Ref	PSD	Verdialona	Solarina	Blanquina	De la Riega	Perico	Durona de Tresali	Raxao	Limón Montés	Xuanina	Regona
Phenolic acids												
AC1(1)	P1	0.08	4.0	7.8	6.3	6.3	7.7	7.3	8.6	5.9	8.5	14.8
AC2(2)	P2	0.21	47.8	49.3	37.7	44.9	46.2	82.1	52.7	44.4	40.5	83.3
Chlorogenic	P3	9.69	512.5	676.4	1023.6	1591.5	835.1	743.1	687.9	893.0	1135.9	852.6
p-Coumaroyl-quinic	P4	0.66	26.2	23.8	7.9	20.3	17.7	52.1	21.0	17.4	10.8	140.4
Coumaric	P5	0.07	5.4	7.5	7.6	8.6	6.4	5.1	5.9	8.2	7.2	9.0
AC3(1)	P6	0.02	6.7	5.2	4.6	4.4	4.7	5.7	4.7	4.4	4.9	5.3
Flavan-3-ols and procyanidins												
Procyanidin B1	P7	1.13	22.6	44.3	13.0	36.0	23.2	31.3	16.6	13.5	21.9	58.0
Flavan-2	P8	0.16	6.8	13.7	4.7	10.9	7.5	10.5	6.6	5.2	6.5	18.6
(+)-Catechin	P9	1.04	30.4	55.7	20.4	45.9	20.6	32.5	19.4	15.0	24.3	100.5
Procyanidin B2	P10	3.00	65.3	140.8	62.5	143.2	99.6	82.8	64.7	64.0	121.5	107.9
(−)-Epicatechin	P11	5.22	162.4	274.0	130.1	273.0	196.6	159.1	128.7	139.6	283.8	241.8
Trimer C1	P12	0.12	4.5	8.2	4.8	7.7	5.8	5.4	4.9	4.9	7.9	6.5
Procyanidin B5	P13	0.20	6.4	11.5	6.2	11.9	9.7	6.7	6.0	6.0	12.0	9.5
Flavan-8	P14	0.03	3.4	2.9	4.7	nd	6.6	nd	3.3	2.8	4.1	2.6
Dihydrochalcones												
DH-1	P15	0.18	15.6	16.8	16.4	15.1	30.2	22.5	36.2	16.3	27.3	17.8
DH-2	P16	0.05	15.2	15.7	16.0	15.7	17.2	18.1	19.5	15.7	17.1	18.6
Phloretin 2′-xyloglucoside	P17	1.29	42.8	65.3	45.9	46.7	237.8	105.2	201.5	50.4	216.1	163.4
DH-4	P18	0.18	15.5	16.7	15.4	15.3	24.6	18.5	22.8	15.3	23.6	19.7
Phloridzin	P19	0.84	41.3	62.6	70.9	67.7	117.2	85.0	105.5	61.6	100.3	237.7
Flavonols												
Hyperin	P20	0.08	7.0	7.6	6.2	7.1	11.4	3.8	7.1	7.4	16.4	4.3
Rutin + Isoquercitrin	P21	0.11	3.9	5.2	4.5	5.9	6.2	4.0	6.0	7.1	5.7	2.8
Reynoutrin	P22	0.06	3.3	5.9	3.5	3.7	5.2	3.5	5.0	7.3	7.8	3.4
Avicularin	P23	0.10	5.4	9.5	5.6	4.9	9.2	5.2	8.8	10.7	15.8	6.0
Quercitrin	P24	0.09	4.5	8.0	9.4	3.9	14.9	3.7	9.1	13.1	7.5	4.0

Values are mean of two determinations; PSD: Pooled Standard Deviation; nd: not detected; (1): *p*-coumaric acic type, $\lambda_{max} = 313$ nm; (2) chlorogenic acid type, $\lambda_{max} = 320$ nm, shoulder at 308 nm.

Table 3
Olfactometric profiles of ice apple juices expressed as mean frequencies (MF, %).

Peak	LRI	Identity	Description	Sig	MF (%)									
					J1	J2	J3	J4	J5	J6	J7	J8	J9	J10
v1	931	Propyl acetate	Fruity	**	25	15	37	0	11	0	0	35	9	0
v2	1057	Ethyl butyrate	Fruity	**	0	0	7	0	0	0	0	52	9	9
v3	1074	Ethyl 2-Methylbutyrate	Fruity, apple	**	33	22	7	9	11	0	36	68	18	18
v4	1299	n.i	Sweet, toasty	***	51	50	0	22	39	30	31	11	55	18
v5	1383	*trans*-3-Hexenol	Grassy	***	9	7	0	0	0	0	0	9	40	0
v6	1404	*cis*-3-Hexenol	Grassy	ns	44	7	7	0	7	7	18	15	25	24
v7	1466	ni	Cooked vegetable	ns	65	44	15	28	47	50	38	63	35	31
v8	1529	*iso*-butyric acid	Fatty	ns	24	29	7	0	20	20	0	9	11	0
v9	1554	1-Octanol	Fatty,waxy	**	0	18	0	0	0	7	0	31	0	0
v10	1635	γ-Butyrolactone	Fatty, waxy	ns	18	18	7	0	24	42	13	24	35	22
v11	1653	ni	Spicy, smoke	***	13	0	7	21	20	0	0	40	0	0
v12	1681	2-methylbutyric acid	Fatty, sweat	**	62	13	0	0	29	54	0	33	57	46
v13	1735	Methionol	Cabbage	*	31	11	0	0	7	0	0	0	13	0
v14	1740	ni	Fruity, sweet	***	0	0	0	0	18	0	0	42	36	35
v15	1775	ni	Aniseed, sweet	ns	35	35	15	22	22	26	22	25	25	18
v16	1837	2-Phenylethyl acetate	Stewed apple	ns	69	66	55	24	72	68	52	49	73	76
v17	1880	Ethyl dodecanoate	Sweet, spicy	**	22	18	0	0	22	37	9	0	31	0
v18	1929	2-Phenylethanol	Roses	**	58	27	24	0	68	30	46	76	62	72
v19	1952	ni	Floral	**	0	0	0	0	24	17	0	0	36	0
v20	1954	ni	Fatty, lactic	ns	0	7	7	0	14	27	0	20	13	33
v21	2001	ni	Phenolic	***	63	29	0	0	18	29	18	38	66	51
v22	2047	Ethyl tetradecanoate	Sweet, candy	ns	61	30	17	9	38	33	9	38	47	0
v23	2078	Octanoic acid	Fatty	***	0	15	7	0	0	0	38	24	0	0
v24	2109	ni	Leather, stable	ns	48	74	43	24	59	46	36	30	35	51
v25	2113	ni	Leather	ns	9	0	17	0	15	0	9	20	33	9
v26	2153	ni	Smoky, leather	**	0	0	0	9	7	0	13	22	18	38
v27	2171	γ-Decalactone	Sweet, spicy	*	31	15	33	0	35	35	0	9	46	46
v28	2183	Eugenol	Spicy	**	24	7	0	0	21	15	51	52	25	35
v29	2210	ni	Curry, spicy	ns	71	71	49	25	53	58	66	73	71	55
v30	2246	4-Vinylguaiacol	Sweet, spicy	***	0	0	7	0	0	7	51	0	31	29
v31	2314	ni	Algae, marine-like	ns	17	42	33	19	35	30	49	30	41	41
v32	2360	Isoeugenol	Spicy	ns	35	7	26	0	27	7	0	33	25	25

LRI: Linear Retention Index; Sig: significance; ns: not significant; (***): significant at 1%; (**): significant at 5%; (*): significant at 10%. J1: Verdialona; J2: Solarina; J3: Blanquina; J4: de la Riega; J5: Perico; J6: Durona de Tresali; J7: Raxao; J8: Limón Montés; J9: Xuanina; J10: Regona.

Table 4
Median values for flavour attributes and quality assessment of the ice apple juices.

	Sig.	Verdialona	Solarina	Blanquina	De la Riega	Perico	Durona de Tresali	Raxao	Limón Montés	Xuanina	Regona
Odour Intensity(1)	ns	1.5 ± 0.1	2.0 ± 0.3	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	1.5 ± 0.0	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	1.5 ± 0.0
Sweetness(1)	**	3.0 ± 0.6	2.0 ± 0.1	2.0 ± 0.3	2.0 ± 0.1	1.5 ± 0.4	2.0 ± 0.3	2.0 ± 0.3	3.0 ± 0.3	1.5 ± 0.4	1.0 ± 0.4
Acidic(1)	***	1.0 ± 0.1	2.0 ± 0.3	2.0 ± 0.4	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.4	3.0 ± 0.3	2.0 ± 0.1	2.5 ± 0.0	2.5 ± 0.1
After-taste persistence(1)	ns	1.5 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.1	2.0 ± 0.0	2.0 ± 0.0
Odour Quality(2)	***	2.5 ± 0.0	3.0 ± 0.1	3.0 ± 0.1	4.0 ± 0.6	2.0 ± 0.3	4.0 ± 0.4	3.0 ± 0.1	2.0 ± 0.9	3.0 ± 0.1	2.5 ± 0.0
Flavour Quality(2)	**	2.5 ± 0.1	3.5 ± 0.5	3.5 ± 0.0	3.5 ± 0.1	2.5 ± 0.0	4.0 ± 0.5	2.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.3	3.0 ± 0.5
Overall assessment(2)	**	2.0 ± 0.4	3.0 ± 0.0	3.0 ± 0.3	3.5 ± 0.1	2.0 ± 0.0	4.0 ± 0.5	2.0 ± 0.6	2.5 ± 0.0	3.0 ± 0.1	3.0 ± 0.5

Data are median ± absolute deviation mean from the median among judges (n = 8); Sig: significance; ns: not significant; (***): significant at 1%; (**): significant at 5%; (1) 3-point scale; (2): 5-point scale.

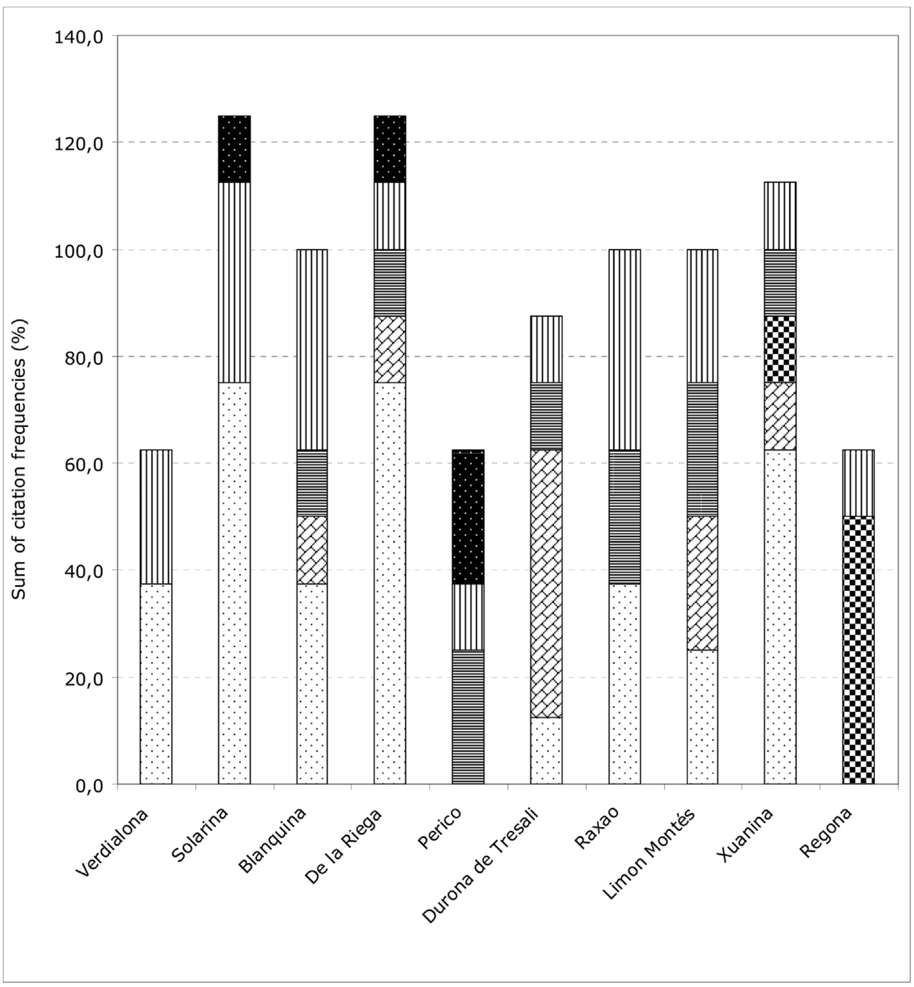

Fig. 1. Aromatic sensory profiles of the cryo-extracted apple juices. Data expressed as citation frequencies (%). References for sensory attributes: Applesauce, ripe apple; Green apple; Tannin/bitter; Grassy; Ripe fruit; Green fruit.

vinylguaiacol, among others, have been associated to the aglycon part of glycosidically bound volatiles in apple fruit (Schwab & Schreier, 1988).

Limón Montés (J8) had the highest contributions of fruity odorants, followed by Xuanina (J9) and Regona (J10), whereas in the juice from Durona de Tresali (J6) cultivar only 2-phenylethyl acetate, with its characteristic applesauce odour, was detected. Regarding the components described as sweet, only the unknown component referred to as v15, having a particular aniseed-like character was detected in all the samples. Ethyl tetradecanoate, previously reported in ciders (Villière, Arvisenet, Lethuaut, Prost, & Sérot, 2012; Williams & Tucknott, 1978) was also present in all the juices excepting that from Regona *cv.* (J10).

Two C6 unsaturated alcohols shared the grassy odour character, namely *trans*-3-hexenol and *cis*-3-hexenol. These components, associated with green notes, have been reported as odour-active in the aroma of apples their levels being influenced by both variety and maturation stage (Mehinagic et al., 2006). These alcohols are synthesized by the action of lipoxygenases (LOX) on substrates like lipids following cellular disruption of apples. Freezing of fruits causes textural losses, inducing cell membrane ruptures with concomitant promotion of enzyme and/or chemical activities. Subzero temperatures delay but do not stop the reactions involved in sensorial quality changes of fruits (De Ancos, Sánchez-Moreno, De Pascual-Teresa & Cano, 2006). The *cis*-isomer was perceived in all the juices except in that from the de la Riega cultivar (J4) whereas the *trans*-isomer was relevant only to the Xuanina (J9) cultivar. The latter variety could be also considered as the most floral, followed by Perico (J5) and Limón Montés (J8). Finally, among the spicy odorants, that referred to as v29 was observed in all the juices.

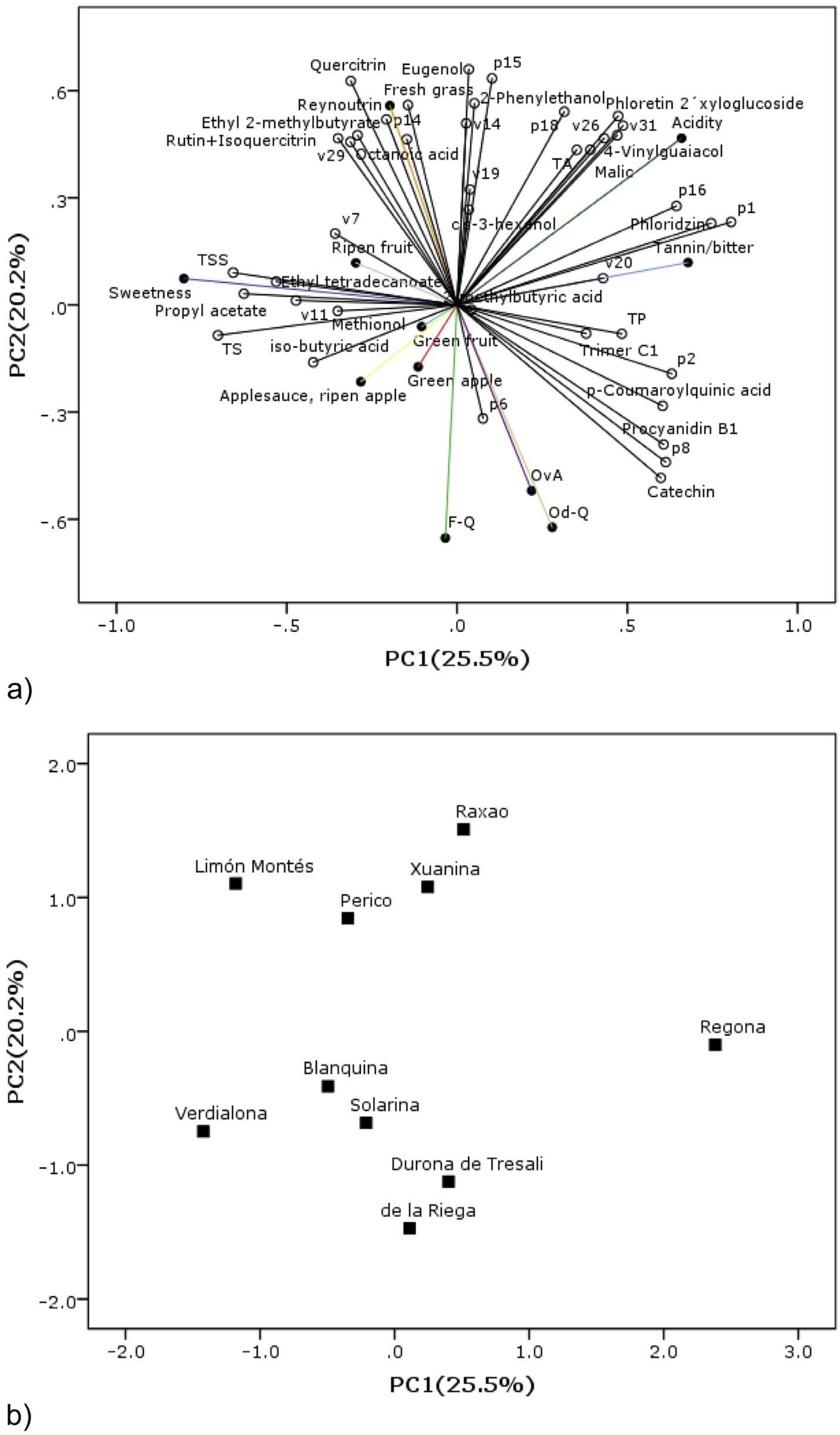

Fig. 2. Projection on the factorial plane formed by the first two principal components.
2a: Chemical, olfactometric and sensory variables; 2b: Cryo-extracted apple juices. Letters *P* or *V* refer to unknown polyphenols or volatiles measured by olfactometry, as seen in Tables 2 and 3.

Eugenol (v28) and isoeugenol (v32) were also widely detected in this set of juices, Verdialona (J1) and Limón Montés (J8) presenting the highest modified frequencies. These phenylpropenes have been reported in fresh apples, their contents increasing during ripening (Atkinson, 2016).

3.4. Sensory description and multivariate analysis

Table 4 summarizes the results for sweetness and acidity intensity and quality of the apple juices. All the samples were assessed as moderate for odour intensity and after-taste persistence; significant differences among apple cultivars were observed for sweetness and acidic perception. The Verdialona cultivar was respectively scored at the high and low extremes of the scales whereas Regona cultivar was the opposite. The other juices were categorised as moderate for both attributes. Regarding quality, the juice from Durona de Tresali was the most appreciated, followed by those from de la Riega, Solarina and Blanquina cultivars.

The aroma profiles of the cryo-extracted juices are shown in Fig. 1. The χ^2 test gave significant differences ($p < .05$) among varieties for all those descriptors excepting applesauce and tannin/bitter. The most cited attribute was "Ripe fruit", present in all the samples evaluated. Solarina, Blanquina and Raxao showed the highest values, followed by Verdialona and Limón Montés cultivars. Next, the applesauce character was identified in almost all of the juices; Solarina, de la Riega and Xuanina showed the highest citation frequencies for this attribute, whereas it was absent in the juices made from Perico and Regona. Grassy and green apple were also highly cited in this set of samples. Perico, Raxao and Limón Montés showed the highest frequency for the grassy attribute and Durona de Tresali the highest for green apple. The high tannin/bitter character shown by the juice made from Regona cultivar is worth noting, this did not compromise its flavour and overall quality assessment, as seen in Table 4.

A categorical principal component analysis was performed to visualize possible relations among different variables. Five components explaining 78% of the variance were computed. Fig. 2a and b represent the projection onto the plane of the first two principal components of variables and samples. As shown in Fig. 2a, the first component is associated with the perception of sweetness, ripe fruit and applesauce/ripe apple (negative loadings), tannin/bitter and acidity (positive loadings). In Fig. 2b the sweetest variety (Verdialona) is placed on the left part of the first axis, and on the opposite side, the juice made from Regona, mainly described by its tannin/bitter character, is projected. Total phenol content and different phenolic compounds like acids referred to as AC1 (p1), AC2 (p2), *p*-coumaroylquinic, dihydrochalcones such as phloridzin, and procyanidin p12, were linked to this attribute. Previous reports on model cider solutions have shown that the perception of bitterness and astringency are influenced by the concentration and the degree of polymerization of procyanidins, although other kinds of phenolic components, such as chlorogenic acid, phloridzin and (−)-epicatechin can elicit an astringent response (Lea, 1990; Naish, Clifford, & Birch, 1993; Symoneaux, Baron, Marnet, Bauduin, & Chollet, 2014a; Symoneaux, Chollet, Bauduin, Le Quéré, & Baron, 2014b). Both bitterness and astringency decrease as sugar concentration increases, whereas the rise in the acid content enhances the perception of astringency (Symoneaux et al., 2014b), which could explain the relative distribution between Verdialona and Regona cultivars. Moreover, not surprisingly relationships were observed between enological parameters and taste or mouthfeel attributes: on the one hand, sweetness and total sugar contents and total soluble solids (ºBrix), associated to Verdialona; and on the other, acidic taste and total acidity and malic acid concentration, were linked to the most acidic cultivars, Raxao and Xuanina.

The second principal component was associated with fruity characters and quality assessments for odour, taste and overall impression (negative loadings), and acid and grassy attributes, with positive loadings (Fig. 2a). The juices made from de la Riega and Durona de Tresali were mainly associated with quality terms, whereas those made from Perico, Raxao, Limón Montés and Xuanina cultivars were linked to the grassy attribute. The samples made from Solarina and Blanquina cultivars were related to green fruit and green apple descriptors (Fig. 2b).

Relationships between odour perception and specific volatile components are usually complex. The fruity character of apples is associated with the presence of ethyl and acetate esters, whereas vegetative descriptors are related to alcohols and farnesene, as shown by Aprea et al. (2012). In Fig. 2a, the grassy attribute was linked to *cis*-3-hexenol and also to components described as spicy (eugenol and v29), floral (2-phenyl ethanol and v19), fruity (ethyl 2-methylbutyrate), sweet (ethyl tetradecanoate) and fatty (octanoic acid), whereas the terms "ripe fruit" and "applesauce-ripe apple" are mainly associated with sweet (ethyl tetradecanoate), vegetal (v7 and methionol), spicy (v11) and fatty odorants, like the *iso*-butyric acid. The relationship between fatty acids and fruity notes has been reported in wines (San-Juan, Ferreira, Cacho, & Escudero, 2011), which illustrates an unexpected interaction among volatile components. Likewise, it should be taken into account that the non-volatile matrix could influence the release of odorants and modify the aromatic perception of the apple juices, as reported for wines elsewhere (Rodríguez-Bencomo et al., 2011; Sáenz-Navajas et al., 2010).

4. Conclusions

The cryo-extraction method is a complex process requiring a strict control of both apple maturity and working temperature during the extraction process to ensure the highest contents of sugars and extraction yields. In this set of Asturian cider apple varieties, and according to the sensory results obtained, Durona de Tresali, de la Riega, Solarina and Blanquina cultivars could be of interest for the making of single-variety ice juices, as they were the most equilibrated and appreciated.

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