Chemical and sensory characteristics of orange based vinegar

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Revised: 21 June 2016 / Accepted: 13 July 2016 / Published online: 18 August 2016
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Abstract Several experiments were conducted to develop orange based vinegar by surface culture. The addition of sugar (sucrose and concentrated must) and the presence/absence of peel in the raw material (squeezed juice, peeled orange, non-peeled orange plus squeezed juice) have been studied during the development of the final product. Polyphenolic and volatile characterization and sensory analysis have also been carried out. The polyphenolic and volatile content of the resulting wines and vinegars showed significant differences depending upon the raw material used. In general, the complexity of the polyphenolic and volatile profiles increased for experiments in which orange peel was included in the raw material. Sensory analysis revealed significant differences between the samples in respect of both sugar addition and raw material. The vinegars using sugar, peeled orange and non-peeled orange plus squeezed juice raw materials, had more preference and keeping in view relative efficiency of the process, vinegar made from the peeled orange material was considered to be best.

Keywords Orange vinegar · Polyphenols · Volatile compounds · Sensory analysis · Characterization

Introduction

Vinegar is a traditional food product produced and consumed in numerous cultures since ancient times, and therefore it is one of the most widespread and well-established products in the human diet all over the world (Solieri and Giudici 2008).

Traditionally, wine vinegar has been the type most frequently commercialized, but in the last few years new products associated with various fruits have appeared on the market, such as vinegars macerated with fruits, fruit juices with vinegar added, and fruit vinegars.

In Asian countries these kinds of vinegar-derived product have been studied for decades. Research studies have been published in Oriental publications on new vinegar products developed with subtropical fruit and other ingredients such as pear (Kim and Lee 2000), persimmon (Kim and Lee 2000; Noda et al. 1991), kiwi (Noda et al. 1991), pumpkin (Liu et al. 2008), tangerine (Tajiri and Fujita 1999), papaya (Wang and Gao 2004), chestnut (Ma et al. 2007) and plum (Zhou and Li 2004); and several patents have been registered.

Although these products have been studied rather less in Western countries, a few publications can be found in the recent literature. For instance, Cejudo et al. studied both the polyphenolic composition and antioxidant activity (Cejudo Bastante et al. 2010) and sensory and volatile profile (Cejudo-Bastante et al. 2013a) of vinegars macerated with orange, lemon, strawberry, grapefruit and lime peel. The same authors studied the sensory profile of new vinegar-derived beverages produced with fruit juices (Cejudo-Bastante et al. 2013b). Úbeda et al. have studied various parameters associated with the production of strawberry and persimmon vinegars such as their composition in major volatile components (Úbeda et al. 2011a),

Electronic supplementary material The online version of this article (doi:10.1007/s13197-016-2288-7) contains supplementary material, which is available to authorized users.

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antioxidant activity and total phenols index (Ubeda et al. 2011b, 2013), and the characterization of active odor compounds (Ubeda et al. 2012). In other research, Hidalgo et al. (2010) have studied the process technology for producing these kinds of fruit-flavored vinegar. Other microbiological and biochemical aspects of such products have also been studied (Budak et al. 2011; Hidalgo et al. 2013a, b). All these research studies indicate an increasing demand for new products of this kind among Western consumers.

In the production of fruit vinegars, several important parameters must be studied in order to obtain an optimum final product. One is the sugar content of the raw material, since this directly affects the acetic acid content of the final vinegar. Another parameter is the presence or absence of fruit peel during the fermentation process, since this will affect the chemical profile of the vinegar produced (Cejudo-Bastante et al. 2010, 2013a).

With respect to orange vinegar, similar products have already been studied in oriental countries such as China, but very little has been published in the international literature concerning this not traditional product (Chang et al. 2005; Tessaro et al. 2010), although orange is a fruit that is consumed very widely as a non-perishable ingredient and flavoring in juices, jams and beverages owing to its high nutritional value and its ready availability in the market.

Orange juice has several beneficial properties. Vitamin C, folate, and other compounds such as carotenoids and phenolic compounds, reduce damage to the DNA and may give protection against mammary, hepatic and colon cancers, and may help prevent degenerative diseases (Tripoli et al. 2007; Rech Franke et al. 2013), such as atherosclerosis and coronary heart diseases (Keli et al. 1996; Steinmetz and Potter 1996). In addition, alcoholic fermentation of orange juice increases its melatonin levels, due to the presence the amino acid precursor, tryptophan, in the composition of the juice. Accordingly, fermented orange juice could be considered a functional food, because melatonin is involved beneficially in various biological processes; this would also be true of orange vinegar (Fernández-Pachón et al. 2014).

According to the latest data from the FAO (FAOStat 2009), in Spain, 4,644,000 tons of oranges and tangerines were produced in 2009, and of this total 334,000 tons (7.4 %) were surplus to market requirements. The development of new products based on oranges, such as orange vinegars, could help to reduce this surplus. Moreover, the market for wine-based vinegars is now saturated with traditional products and so the production of orange vinegar could be a useful product diversification for manufacturers.

In the research described here, an orange-based vinegar has been developed and characterized. To our knowledge, this is the first attempt that such a vinegar has been

developed and characterized for volatile and polyphenolic compounds. Finally sensory analysis has been employed to assess the influence of the various parameters on the final product.

Materials and methods

Raw material

Oranges supplied by a local company were thoroughly washed and dried, in order to decrease any possible traces of pesticides. They were then processed to obtain three types of starting matrices: squeezed whole orange juice (hereafter referred to as SJ), crushed peeled orange without the outer zest (flavedo) but with the pith (albedo) (referred to as PO), and crushed whole (non-peeled) orange + orange juice 50 % (w/v) (NPO + SJ). The oranges were crushed mechanically using a conventional beater. The finished juice showed a Brix value around 11 °Brix, and a total acid content of between 5 and 8 g tartaric acid/L. With the aim of studying the contribution of the sugar content to the properties of the final vinegar, some of the raw material samples were sweetened to 14–15 °Brix, using either sucrose or concentrated must (Agrovin, Ciudad Real, Spain). Therefore, taking into account the different processes applied to the fruit, a total of 9 different starting matrices were fermented, in duplicate.

Wine making

Laboratory-scale alcoholic fermentations were carried out using 3 L-glass vessels. 60 mg/L of sulfur dioxide (from potassium metabisulfite, Agrovin) was added to prevent the growth of undesirable microorganisms. 3 mL/L of pectolytic enzymes (Enartis Zym RS, Trecate, Italy) was then added to the matrix as clarifying agent; and 35 g/hL of diammonium phosphate (Actimax Plus+, Agrovin) was added as a nutrient. Fermentation was performed by inoculating 20 g/hL of *Saccharomyces bayanus* active dry yeast (Enartis Ferm SB, Trecate, Italy) into the starting matrix. The yeast was previously activated at 35 °C during 20 min. Fermentation ended when the Brix value was around 1 °Bx. The orange wine samples were then centrifuged at 15,000g for 10 min and stored at 4 °C until used.

Vinegar making

Preparation of the starting culture

Unfiltered Sherry vinegar (400 ml) was centrifuged at 10,000g for 5 min at 4 °C to obtain the acetic acid bacteria

starting culture or inoculum. This was then dissolved in 400 mL of the orange wine and subjected to aeration (7.5 L/h L), under controlled conditions of temperature (29 °C) to allow the population of acetic acid bacteria to multiply. The acetic acid bacteria were grown in a Frings Acetator (Heinrich Frings, Bonn, Germany). When the inoculum had been prepared, it was added to the vessels containing the orange wines, to carry out the acetic fermentation. The inoculum volume was 16 % of the total volume contained in each vessel.

Acetic fermentation

Acetic fermentation was carried out by surface culture in glass vessels of 3 L capacity. If the acetator was used, to change from some conditions to others, the process had to be stopped every time, with the subsequent period of time needed for bacteria to restart the fermentation. This fact was not operative so, surface culture instead of submerged culture was used to optimize the fermentation conditions (9 conditions in duplicate carried out at the same time). The fermentation process was stopped when the acetic acid content was stable for a period of 7 days. The resulting orange vinegars were stored at 4 °C until analysis.

Analysis of phenolic compounds

UPLC separation and identification of phenolic compounds were performed using a Waters Acquity UPLC system (Waters Corps. Milford, MA, USA), equipped with a diode array detector (DAD). In accordance with the method proposed by Schwarz et al. (2009), an Acquity UPLC BEH C18 column (100 × 2.1 mm/ID, with 1.7 µm particle size), also from Waters, was used. The column temperature was maintained at 47 °C. The binary system phases were: A (3 % acetonitrile, 2 % acetic acid, 95 % water) and B (85 % acetonitrile, 2 % acetic acid, 13 % water), with a flow rate of 0.7 mL/min, giving a maximum back pressure of 10.400 psi, which is within the capabilities of the UPLC. The injection volume was 2.5 µL. The 6.5 min gradient was as follows: 0 min, 100 % A, 3 min, 90 % A, (curve 6) 4 min, 90 % A, 6.5 min, 25 % A (curve 6). Finally, the column was washed with 100 % B for 3 min and equilibrated with 100 % A for 3 min. All the samples were previously filtered through 0.45 and 0.22 µm nylon filters, from Scharlab (Barcelona, Spain).

The compounds were identified and quantified using the diode array detector chromatograms obtained at 280 and 320 nm, by comparing retention times and UV–VIS spectra with those provided for commercial standards [gallic acid, narirutin, naringin, hesperidin, neohesperidin, sinensetin, nobiletin (280 nm), chlorogenic acid, caffeic acid, *p*-

coumaric acid, ferulic acid (320 nm)]. All analyses were performed in duplicate.

Analysis of volatile compounds

Verification of the extraction method

In order to verify if the optimized method proposed by Guerrero et al. (2006) was acceptable for all the samples (juices, wines and vinegars), model solutions with different amounts of sugar, ethanol and acetic acid were formulated and analyzed. Model juices with 100 and 130 g/L of sucrose, model wines with 5 and 7 g/L of ethanol, and model vinegars with 4 and 6 g/100 mL of acetic acid were prepared. All the solutions contained 0.5 g/100 mL of ascorbic acid, 3.8 g/L of citric acid, a pH value of 3.7, 60 mg/L of sulfur dioxide, and 8 volatile compounds typical of orange (Fan et al. 2009) belonging to different families of chemicals: (hexanal, isoamyl acetate, hexanoic acid, 1-hexanol, diethyl succinate and benzaldehyde at 0.5 mg/L, and D-limonene and 2-phenylethanol at 1 mg/L). All analyses were performed in duplicate.

The standard compounds were supplied by Sigma (Steinheim, Germany).

Sample preparation

For preparing the sample, commercial polydimethylsiloxane stir bars, of 10 mm length × 0.5 mm film thickness (supplied by Gerstel, Mülheim a/d Ruhr, Germany), were used for the extractions. In accordance with the method proposed by Guerrero et al. (2006), for each stir bar sorptive extraction (SBSE) analysis, a volume of 25 mL of sample was pipetted and placed into a 100-mL Erlenmeyer flask with 5.85 g of NaCl (Scharlau, Barcelona, Spain) and 84 µL of a solution of 4-methyl-2-pentanol (Sigma, Steinheim, Germany) (2.27 g/L in Milli-Q water containing 80 g/L of acetic acid). The Erlenmeyer flask was placed on a 15-position magnetic stirrer (Gerstel). Later, the stir bar was rotated at 1250 rpm at 25 °C for 120 min. After removal from the vinegar sample, and in order to eliminate NaCl and any remains of orange pulp attached, the stir bar was placed for a few seconds in distilled water and then gently dried with a lint-free tissue. Then, it was transferred into a glass thermal desorption tube and thermal desorption was carried out.

The system used for the thermal desorption of the coated stir bars was a commercial TDS-2 thermal desorption unit (Gerstel) connected to a CIS-4 programmed-temperature vaporization (PTV) injector (Gerstel) by a heated transfer line. The PTV was installed in an Agilent 6890 GC-5973 MS system (Agilent Technologies, Palo Alto, CA, USA).

An empty baffled liner was used in the PTV. The thermodesorption unit was equipped with a MPS 2 L autosampler (Gerstel) capable of handling the program for 98 coated stir bars. Desorption temperature was programmed from 40 to 300 °C (held for 10 min) at 60 °C/min under a helium flow (75 mL/min) and the desorbed analytes were cryofocused in the PTV system with liquid nitrogen at −140 °C. Finally, the PTV system was programmed from −140 to 300 °C (held for 5 min) at 10 °C/s for analysis by gas chromatography–mass spectrometry (GC–MS). An Agilent 6890 GC-5973 N MS system (Agilent, Little Falls, DE, USA), equipped with a DB-Wax capillary column (J&W Scientific, Folsom, CA, USA), of 60 m × 0.25 mm i.d., with a 0.25 µm coating, was used to perform the capillary GC–MS analysis in the electron impact mode. The carrier gas was helium at a flow rate of 1.0 mL/min. Peak identification was carried out using the Wiley library by analogy of mass spectra and confirmed by retention times of standards, when they were available, or by the retention data from the literature. Semi-quantitative data were obtained by measuring the relative molecular ion peak area in relation to that of 4-methyl-2-pentanol, the internal standard. For all the compounds, the retention indices were determined (on a DB-Wax polar column) and compared with those from the literature. All analyses were performed in duplicate.

Sensory analysis

The evaluation sessions were carried out in a standard tasting room (UNE 87-004). The panel of 10 judges, all of whom were laboratory personnel, had previously undergone training regarding general and specific aspects of sensory evaluation. The judges rated the orange wines and vinegars on a structured nine-point scale (UNE 87020 equivalent to ISO4121:1987), evaluating descriptors such as general impression, mouth feel, flavor intensity, orange-like quality, fruitiness and pungency as positive attributes, and wine character, yeast, and defaults considered as negative attributes. Descriptors were quantified as follows, by: absence (1), weak (3), moderate (5), pronounced (7) or highly pronounced (9). Samples were presented in wine glasses and covered with a glass top in order to minimize possible decreases of aroma intensity.

Statistical analysis

Data were subjected to an Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and Cluster Analysis (CA), using the statistical computer packages Statgraphics Centurion XVI (Statpoint Technologies, Inc., USA), and SPSS Statistics version 17.0 (IBM Corp., NY, USA).

Results and discussion

Orange wine and vinegar production

Alcoholic fermentation

Alcoholic fermentation of the squeezed juice (SJ) and peeled orange (PO) samples was completed in 4 days. The ethanol content was around 5 % in non-sweetened samples of SJ and PO, and around 7 % in sweetened samples. Alcoholic fermentation was inefficient in the samples of non-peeled orange plus juice (NPO + SJ). Non-sweetened samples NPO + SJ took 30 days to complete the alcoholic fermentation, and only reached an alcoholic degree value of less than 2 %, probably due to the rheology of the product or to the presence of essential oil components which might possess antimicrobial effect. In the sweetened samples of NPO + SJ the transformation from sugar to ethanol did not take place at all, so those samples were eliminated from the study.

Acetic fermentation

The acetic fermentation experiments were completed after periods of between 24 and 30 days after inoculation. The final acidity of samples was around 4 g/100 mL of acetic acid except for those from the NPO + SJ material, which reached a value of less than 2 g/100 mL of acetic acid. The alcohol content of the wines obtained was around 5 or 7 % in some cases. Taking into account that the surface culture method of fermentation has not an efficiency of 100 %, the obtained values of acetic acid content were in the range of the expected ones.

Process efficiency

The final products were centrifuged and the solid parts were removed. A higher or a lower volume of liquid of the final product was recovered depending on its rheology. Therefore, taking this into account, different efficiencies of the process were achieved. The efficiency percentages achieved were as follows: SJ samples, 60 %; PO samples, 40 %; and NPO + SJ samples, 20 %; this last is considered to be an unacceptably low efficiency.

Phenolic compounds

A total of 26 polyphenolic compounds were studied in the samples of orange juice, wine and vinegar. Among the compounds identified in these samples were those derived from hydroxycinnamic and hydroxybenzoic acids, such as ferulic acid, *p*-coumaric acid, gallic acid, caffeic acid, and

chlorogenic acid, and flavanones such as hesperidine, neohesperidine, narirutin and naringin. These were the most important phenolic compounds typically found in orange juices (Kelebek et al. 2009; Destani et al. 2013). Two polymethoxylated flavones, sinensetin and nobiletin, were also identified in the samples; these have also been found previously in citrus samples (Tripoli et al. 2007; Yu et al. 2014; Donato et al. 2014; Zhang et al. 2013).

Data were subjected to Analysis of variance (ANOVA). Three factors were considered: matrix (juice, wine and vinegar), raw material (SJ, PO, NPO + SJ) and added sugar (no addition, added sucrose and added concentrated must). The results obtained showed significant differences ($p < 0.01$) according to matrix (juice, wine and vinegar), and raw material (SJ, OP and NPO + SJ). The factor of added or non-added sugar (sucrose or concentrated must) was not significant for the majority of the compounds studied (Supplementary table 1). For each significant factor, Tukey's test was employed for the comparison of means and the results are shown in Supplementary table 2. It is worth to mention that some compounds such as the polymethoxylated flavanones nobiletin and sinensetin, were only present in juice, wine, and vinegar samples derived from PO and NPO + SJ raw materials, but not in those derived from SJ. Compounds of this type were characteristic of the solid parts of orange (Li et al. 2006; Yu et al. 2014).

To analyze results on a multivariate basis, principal component analysis (PCA) was carried out on all samples. Applying the Kaiser criterion (eigenvalue > 1), 5 PCs were obtained which explained 91 % of the total variability of the samples. Attending to only the first two components, 72.2 % of total variance could be explained. The first component (PC1) was related to flavanones, which had the most influence on the variability of the data, and the second (PC2) was related to hydroxycinnamic acid derivatives. Figure 1a shows the distribution of the samples on the plot defined by the first two components. As can be seen, three groups are obtained depending on the raw material: SJ, PO and NPO + SJ. As mentioned above, the polyphenols with the greatest positive influence on PC1 were flavanones. The distribution of samples obtained reflects a higher content of these compounds for those samples from the NPO + SJ material.

On the basis of the results obtained, the type of initial raw material was the variable with the most influence on the polyphenolic composition of the samples.

Volatile compounds

In order to verify if the optimized extraction method proposed by Guerrero et al. (Guerrero et al. 2006) was acceptable for the different samples studied (juices, wines

Fig. 1 Principal components analysis. Distributions of samples on the plane defined by the first two principal components. **a** Polyphenols. 0 Samples from squeezed juice (SJ); 1 Samples from peeled orange (PO); 2 Samples from non-peeled orange + squeezed juice (NPO + SJ). **b** Volatile composition. 1 juices, 2 wines, 3 vinegars

and vinegars), model solutions with different amounts of sugar, ethanol and acetic acid were formulated and analyzed. These preliminary studies showed no significant differences in the determination of volatile compounds in different matrices, and RSD values for the different volatile compounds considered were lower than 10 %. Therefore the same method was applied to the different types of sample analyzed: orange juice, orange wine and orange vinegar (data not shown).

A total of 35 volatile compounds, belonging to several different families, were identified (Table 1).

In order to determine possible statistical differences between the samples in the composition of volatile compounds, data were subjected to analysis of variance (ANOVA), taking into account three independent factors (type of matrix, raw material and added sugar). Fisher's weight and p value was calculated to establish the discriminant capacity of each variable (Supplementary table 3).

As previously observed for polyphenolic compounds, the two factors, matrix and raw material, have a significant influence on the volatile composition of the samples ($p < 0.01$), but not the addition or non-addition of sugar, in general terms (Supplementary table 3).

Table 2 shows mean relative areas for the different studied volatile compounds, according to type of matrix

Table 1 Volatile compounds identified by SBSE–GC–MS

Compound	LRI	Descriptor
Ethyl butanoate	1030	Fruit, pineapple, orange
Hexanal	1094	Herbaceous
Isoamyl acetate	1120	Banana, blueberry, strawberry
D-Limonene	1182	Orange, mint, lemon, floral
1-Hexanol	1344	Floral, grass
3-Hexen-1-ol	1345	Herbaceous
Ethyl octanoate	1414	Strawberry, banana
Decanal	1479	Citrus
Benzaldehyde	1489	Blueberry, fruit
Linanol	1547	Floral, orange, rose, citrus, sweet
1-Octanol	1575	Floral, herbaceous, orange leave
4-Terpineol	1613	Sweet, floral
Isovaleric acid	1690	Cheese
Ethyl decanoate	1701	Fruit, cognac
Diethyl succinate	1711	Winy
Hexanoic acid	1722	Boiled vegetable, potato, cheese
α -Terpineol	1767	Apple, orange leave, mandarin, lemon
Valencene	1806	Wood, citrus, orange
β -Citronelol	1825	Floral, sweet, fruit, soapy
Nerol	1836	Floral, orange blossom
<i>trans</i> -Carveol	1860	Mint, green
Geraniol	1879	Floral, rose, green, sweet
<i>cis</i> -Carveol	1899	Mint, green
Benzyl alcohol	1912	Fruit, soft, sweet, metallic, alcohol
2-Phenylethanol	1944	Floral, rose, honey
β -Ionone	1984	Blueberry, strawberry
Octanoic acid	2101	Sour, fat, butter, chemical
Nonanoic acid	2200	Cheese, soapy
4-Vinylguaiacol	2215	Clove, vanilla, pepper
β -Farnesene	2220	Green apple
Decanoic acid	2317	Chamomile, sweet, floral
β -Humulene	2422	Hop, coriander
Nootkatone	2517	Grapefruit

LRI Linear Retention Index

(juices, wines and vinegars) and results from Tukey's test. RSD values are calculated taking into account all the samples studied: 9 experiences (SJ, PO, NPO + SJ, and the use or not of sucrose or concentrated must) in duplicate and taking into account that all of them were analyzed in duplicate, i.e.: RSD values derived from 36 analyses as maximum for each matrix (juice, wine, vinegar). The different raw materials employed for each matrix would explain the high RSD values obtained and therefore the high variability among the samples.

Alcoholic fermentation increased significantly the concentration of volatile compounds except for terpenes, sesquiterpenes and aldehydes, which are considered primary aromas given off by the original fruit. However, the process of acetic fermentation reduced the content of all

families of volatile compounds, with the exception of some acids and aldehydes (Table 2).

Volatile compounds were also submitted to principal component analysis (PCA). In this case, 7 PCs were obtained according to the Kaiser criterion (eigenvalue > 1). The first two PCs together explain 54.17 % of the variability of the samples. The first PC is related mainly to the presence of terpenols, which come from the initial juice. The second PC is related to fermentation-derived compounds, such as esters, alcohols and benzenic compounds. Figure 1b shows the distribution of all samples on the plane defined by these two PCs. As can be seen, for volatile compounds, no clear separation of samples is obtained; however, two groups may be identified, one corresponding to the wine samples, and the other to the

Table 2 Mean relative areas of the volatile compounds studied in the different matrixes

Volatile Compounds	Orange juices		Orange wines		Orange vinegars	
	Mean	RSD	Mean	RSD	Mean	RSD
Ethyl butanoate	0.052 ^a	65.3	0.031 ^a	70.3	ND ^b	–
Hexanal	0.005 ^a	182.3	ND ^b	–	ND ^b	–
Isoamyl acetate	0.035 ^a	65.4	1.332 ^b	83.8	0.034 ^a	228.1
D-Limonene	19.035 ^a	38.7	4.720 ^b	150.5	0.021 ^c	146.5
1-Hexanol	0.025 ^a	131.2	0.092 ^b	51.2	ND ^a	–
3-Hexen-1-ol	0.005 ^a	50.9	0.009 ^a	60.5	ND ^b	–
Ethyl octanoate	0.023 ^a	47.8	1.361 ^b	90.2	0.033 ^a	191.6
Decanal	0.014 ^a	125.2	0.010 ^a	158.9	0.015 ^a	64.1
Benzaldehyde	0.002 ^a	97.5	0.002 ^a	206.6	0.002 ^a	65.9
Linalol	2.515 ^a	145.0	3.909 ^a	96.4	0.008 ^b	89.3
1-Octanol	0.209 ^{a,b}	166.9	0.473 ^a	136.1	ND ^b	–
4-Terpineol	2.558 ^a	157.1	3.248 ^a	114.8	0.074 ^b	95.1
Isovaleric acid	0.009 ^a	69.8	0.043 ^b	61.9	0.091 ^c	37.8
Ethyl decanoate	ND ^a	–	0.124 ^b	109.3	0.010 ^a	259.5
Diethyl succinate	ND ^a	–	0.004 ^{a,b}	153.3	0.009 ^b	88.2
Hexanoic acid	0.017 ^a	136.3	0.095 ^b	53.0	0.126 ^b	43.4
α -Terpineol	1.375 ^{a,b}	174.5	1.916 ^a	132.0	0.265 ^b	191.5
Valencene	0.254 ^a	101.3	ND ^b	–	ND ^b	–
β -Citronelol	0.063 ^{a,b}	165.8	0.308 ^a	99.8	ND ^b	–
Nerol	0.169 ^{a,b}	132.2	0.288 ^a	91.9	0.005 ^b	67.3
<i>trans</i> -Carveol	0.059 ^a	121.6	0.091 ^a	63.3	0.104 ^a	59.8
Geraniol	0.298 ^{a,b}	159.1	0.447 ^a	119.1	0.011 ^b	110.8
<i>cis</i> -Carveol	0.012 ^a	100.9	0.019 ^a	53.2	0.014 ^a	71.9
Benzyl alcohol	0.053 ^a	141.3	0.724 ^b	62.6	0.315 ^a	158.9
2-Phenylethanol	0.053 ^a	141.5	0.731 ^b	62.4	0.317 ^a	158.8
β -Ionone	0.018 ^a	77.7	0.058 ^b	81.2	ND ^a	–
Octanoic acid	0.127 ^a	115.9	1.527 ^b	51.0	1.853 ^b	47.4
Nonanoic acid	0.006 ^a	103.5	0.020 ^a	25.5	0.072 ^b	68.6
4-Vinylguaiaicol	0.030 ^a	138.9	0.150 ^a	96.5	0.162 ^a	56.4
β -Farnesene	0.021 ^a	141.4	ND ^b	–	0.005 ^b	86.1
Decanoic acid	0.017 ^a	63.3	0.494 ^b	69.9	0.260 ^a	51.6
β -Humulene	ND ^a	–	0.007 ^b	56.8	0.008 ^b	62.5
Nootkatone	0.384 ^a	73.6	2.276 ^b	96.7	1.019 ^a	81.9

For each row, different superscripts indicate significant differences at $p < 0.01$ (Tukey's test)

RSD relative standard deviation, ND not detected

vinegar samples and juice samples. The samples corresponding to vinegars are not clearly separated from the juice samples, possibly due to the decrease of several alcoholic fermentation-derived compounds during the acetification process (Table 2).

Finally, both polyphenols and volatile compounds were jointly considered and submitted to a hierarchical agglomeration cluster analysis (Fig. 2). The squared Euclidean distance as metric and the Ward method as the amalgamation rule were taken into account.

As can be seen, four groups were obtained: juices, wines and vinegars from squeezed juices (SJ), wines and vinegars from peeled oranges (PO), and wines and vinegars from non-peeled oranges + squeezed juices (NPO + SJ). This grouping showed that the most significant factor in the analytical data (polyphenols and volatile compounds) was type of raw material, with those wines and vinegars from non-peeled oranges exhibiting a more distinctive polyphenolic and volatile profile.

Fig. 2 Cluster analysis taking into account composition of polyphenolic and volatile compounds of juice, wine and vinegar.

Sensory analysis of wine and orange vinegar

Sensory data were also submitted to ANOVA and PCA. Taking into account the considerable sensory difference existing between a vinegar matrix and a wine matrix, sensory data were studied separately. So, in this case, the factors considered for the ANOVA were type of raw material and added sugar.

Bitterness is one of the main problems of citrus juices. However it was not significant in the sensory analysis for the majority of the samples so it was not taken into account in the study. Just those samples derived from NPO + SJ presented this attribute, so a relationship between bitterness and the employment of peel could be observed. In addition, fermentation step seems to eliminate this problem for the rest of the samples. Further studies regarding this aspect should be carried out in the future.

Orange wine

Analysis of variance showed significant differences according to raw material ($p < 0.01$). The added sugar factor did not show significant differences between the samples.

PCA revealed two principal components according to the Kaiser criterion (eigenvalue > 1). These two components together explained 65.86 % of the total variance of the samples. Figure 3a shows the scattering of the samples on the plot defined by the first two components and the loading of the descriptors considered. Desired descriptors (fruit, aromatic intensity, orange, general impression) showed a higher loading on the first component (PC1), whereas undesired descriptors (wine character, yeast) had a higher loading on the second component (PC2).

The best rated wines were made from NPO + SJ or PO, with or without sugar added. However, from a technological point of view, NPO + SJ procedure provided a low efficiency.

Orange vinegar

For vinegars, analysis of variance revealed significant differences for both variables: type of raw material and added sugar ($p < 0.01$).

Principal component analysis allocated descriptors in two groups. Negative values of PC1 were related to undesired descriptors (yeast, lactic character, defects), and positive values of PC1 were related to desired ones

Fig. 3 Sensory analysis. principal components analysis. Distribution of the samples (**a** wines, **b** vinegars) on the plane defined by the first two components, and loadings of the different descriptors on these two PCs. **a** Orange wine. 1 Wines from squeezed juice. 2 Wines from Non-Peeled orange + squeezed juice. 3 Wines from Peeled Orange. **b** Orange vinegar. Vinegars from all three raw materials were considered (squeezed juice, non-peeled orange + squeezed juice and peeled orange) and with: 1 no sucrose added. 2 Addition of concentrated must. 3 Addition of sucrose

(orange, aromatic intensity, fruitiness, pungency). As can be seen in Fig. 3b, samples with added sugar were situated around positive values of PC1.

Conclusion

The different profiles of polyphenolic and volatile compounds have been obtained depending on the type of raw material (SJ, PO, NPO + SJ) and matrix (juices, wines, vinegars), having sugar addition in any form did not effect significantly. The type of raw material has been demonstrated to have the most influence on analytical and sensory outcomes. The optimum experimental condition for obtaining orange vinegar required the use of PO or NPO + SJ as raw material, with the associated content of

solid parts of the orange that has a significant influence on the composition of the product obtained. In the case of NPO + SJ samples, the low efficiency of the process must be considered a serious disadvantage. In respect of sensory qualities, the initial raw material should be sweetened in order to obtain optimum flavor in the orange vinegar.

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