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CHARACTERIZATION OF ODOUR-ACTIVE VOLATILE COMPOUNDS OF ACEROLA WINE

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The volatile compounds of acerola wine were isolated by headspace–solid phase microextraction (HS-SPME) and analysed by gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS), and gas chromatography-olfactometry (GC-O). The composition of acerola wine included 38 esters, 19 alcohols, 16 acids, 8 terpenes, 5 aldehydes, 5 ketones, 3 furans, and 8 miscellaneous compounds. The odour-active compounds were screened by application of the aroma extract dilution analysis and odour activity values. Nineteen odorants were considered as odour-active volatiles, from which methyl 2-methylbutanoate and 2-ethylhexan-1-ol were the most odour-active compounds.

Keywords: *Malpighia glabra*, acerola, wine volatiles, HS–SPME, GC-O, odour activity value

Acerola (*Malpighia glabra* L.) is a minor non-conventional fruit cultivated in many tropical zones. The fruit is considered as an excellent source of antioxidant and vitamin C, and it is consumed fresh and used in food industry to produce juices, jams, and beverages (Soares Filho & Oliveira, 2003). Although the better-appreciated wines are made from grapes, other fruits could be utilized as raw materials for the manufacture of wines, such as acerola (Almeida et al., 2010, 2014; Minh, 2015). These wines have flavour and aroma characteristics of the original fruit and a good acceptance by the consumers. Although volatile compounds of acerola fruit have been studied to some extent (Vendramini & Trugo, 2000; Boulanger & Crouzet, 2001; Pino & Marbot, 2001), there is no information published to date on the volatiles of acerola wine.

Because the knowledge on the key odorants in the final product is the prerequisite for investigation on the influence of processing steps, the aim of the present study was to determine aroma profile and odour-active compounds of acerola wine by application of the aroma extract dilution analysis (AEDA) and odour activity values (OAV).

1. Materials and methods

Chemical standards ethyl propanoate (99%), 3-methylbutan-1-ol (>99%), methyl 2-methylbutanoate (99%), ethyl butanoate (99%), ethyl pyruvate (98%), 2,3-butanediol (98%), ethyl 2-methylbutanoate (99%), ethyl 3-methylbutanoate (98%), 3-methylbutyl acetate (>99%), 5-methyl-2-furfural (99%), ethyl hexanoate (>99%), hexyl acetate (99%), 2-ethylhexan-1-ol (99%), allyl hexanoate (98%), 2-phenylethanol (>99%), ethyl

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3-phenylpropanoate (97%), and methyl nonanoate (98%) were purchased from Sigma–Aldrich (St. Louis, MO). A C_8–C_{32} *n*-alkane mixture, used for determination of Kovats retention indices were obtained from Sigma–Aldrich (St. Louis, MO). Absolute ethanol (>99.5%), anhydrous citric acid (99%), and sodium chloride (99.5%) were purchased from Merck (Darmstadt, Germany).

Fresh and ripe acerola fruit (25 kg) were passed through a colloid mill. The pulp was added at 10% (w/w) to a wort containing brown sugar (190 g l^{-1}), dibasic ammonium phosphate (1 g l^{-1}), and anhydrous citric acid (2 g l^{-1}). The wort was transferred into a stainless-steel tank for the fermentation using dried *Saccharomyces cerevisiae* yeasts (1 g l^{-1}, Fermipan Lefersa, Havana). Fermentation was performed in duplicate at controlled temperatures (26±2 °C). After fermentation, the wine was racked by adding 0.5 g l^{-1} sodium bisulphite and clarified by adding 0.1 g l^{-1} kieselguhr. After 7 days, the wine was decanted and it was stored at 25 °C for one month. The wines were packed in amber-coloured bottles of 0.75 l, which were sealed with cork. The wine was pasteurized by heating in a closed pan at 60 °C for 15 min, cooled later in running water, and stored in refrigerator at 5 °C for a period of three months for posterior evaluation of its quality. The general compositions of juice and wine are given in Table 1.

Table 1. General composition of acerola juice and wine

Juice composition	
Soluble solids (°Brix)	6.5±0.1
Total acidity (g l^{-1} as anhydrous citric acid)	7.0±0.6
pH	3.60±0.01
Wine composition	
Alcohol (% v/v)	11.40±0.01
Total acidity (g l^{-1} as anhydrous citric acid)	2.3±0.2
pH	3.12±0.01
Ash (g l^{-1})	0.90±0.07

Brix value (method 932.12), total acidity (method 942.15), and pH (method 981.12) were determined in acerola juice, while alcohol (method 969.12), total acidity (method 962.12), and pH (method 960.19) in wine according to standard methods (AOAC, 2012).

Considering previous experiences (Pino & Queris, 2010), the fibre used was coated with polydimethylsiloxane (PDMS), 100 µm film thickness (Supelco, Bellefonte, PA). The fibre was thermally conditioned in accordance with the manufacturer's recommendations before first use. HS-SPME extractions were carried out by placing 8 ml of wine, 1 g of NaCl, and 20 µl of a methyl nonanoate internal standard solution (20 mg l^{-1} in ethanol) into a 15 ml-vial sealed with a PTFE/Silicone septum (Supelco, Bellefonte, PA). The mixture was carefully shaken and then left to equilibrate 15 min before the analysis. The results obtained from a previous work (Pino & Queris, 2010) showed that 30 °C headspace sampling temperature and 30 min extraction time under stirring mode (500 min^{-1}) resulted in the highest extraction efficiency.

GC-FID analysis was performed on a Konik 4000A instrument (Konik, Barcelona) using hydrogen as the carrier gas at 1 ml min^{-1}. Columns used were DB-Wax (30 m × 0.25 mm, 0.25 µm film thickness; J & W Scientific, Folsom, CA) or DB-5ms (30 m × 0.25 mm, 0.25 µm film thickness; J & W Scientific, Folsom, CA), working with the following temperature program and conditions: 50 °C for 2 min, ramp of 4 °C min^{-1} up to 250 °C; injector and detector temperatures 250 °C; detector FID; splitless injection (straight glass liner, 0.75 mm I.D.) for 2 min. The relative quantities of the volatiles were expressed as peak area per cents in the GC-FID chromatogram. For some compounds (ethyl propanoate, 3-methylbutan-1-ol, methyl 2-methylbutanoate, ethyl butanoate, ethyl pyruvate, 2,3-butanediol, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, 5-methyl-2-furfural, ethyl hexanoate, hexyl acetate, 2-ethylhexan-1-ol, allyl hexanoate, 2-phenylethanol, and ethyl 3-phenylpropanoate), chemical aroma standard mixtures were prepared in an 11% (v⁄v) hydro-alcoholic solution to bracket the concentrations of each individual compound in acerola wine. Standard curves according to the internal standard method were created for these compounds. All analyses were replicated three times.

GC-MS analysis was made on a HP-6890 instrument gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) interfaced with a HP-5973 mass-selective detector fitted with a DB-5ms column (30 m × 0.25 mm, 0.25 µm film thickness; J & W Scientific, Folsom, CA). Analytical conditions were the same as for GC-FID analyses: injector and transfer line temperatures 250 °C; carrier gas helium at 1 ml min^{-1}; splitless injection (straight glass liner, 0.75 mm I.D.) for 2 min. Mass spectra in the electron impact mode (EI-MS) were generated at 70 eV and acquisition was performed in scanning mode (mass range m⁄z 35−400 u). Identification of the constituents was based on comparison of the linear retention times with those of authentic samples, comparing their linear retention indices relative and on computer matching against commercial libraries (NIST 02, Wiley 275, Palisade 600, and Adams 2001) and FLAVORLIB homemade library mass spectra built up from pure substances and components of known essential oils. Some of the identifications were confirmed by the injection of the chemical standards into the GC-MS system. Linear retention indices of the compounds were calculated using an *n*-alkane series.

GC-O and AEDA analyses were performed on a HP-6890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) converted for GC-O use by installing a glass effluent splitter and a glass sniffing port. The DB-5ms capillary column was connected to the glass effluent splitter with two deactivated fused silica tubing outlets of equal lengths (50 cm × 0.25 mm) conducting the column effluent to the FID and to the sniffing port. Analyses were carried out using the analytical parameters described above for the GC analyses.

An approach of the AEDA technique developed for GC-O analysis of wine (Martí et al., 2003) to estimate the sensory contribution of each odorant was used in the present study. It consists of carrying out successive dilutions of the acerola wine (steps 1:4) with a synthetic wine before the SPME. A mimic matrix was prepared by dissolving 2.3 g anhydrous citric acid and 114 ml of absolute ethanol in a suitable amount of Milli-Q water to give one litre of solution. The pH value was adjusted to 3.1 with 0.1 N NaOH. To check the linearity of the HS-SPME procedure, a model mixture was prepared by adding some volatile compounds (ethyl propanoate, 3-methylbutan-1-ol, ethyl 3-methylbutanoate, 5-methyl-2-furfural, ethyl hexanoate, 2-ethylhexan-1-ol, 2-phenylethanol, and ethyl 3-phenylpropanoate), in a concentration level like acerola wine, to the synthetic wine solution.

The HS-SPME extracts from wine and its successive dilutions (1:4) were analysed using the methodology described earlier (Martí et al., 2003). The flavour dilution (FD) factors

obtained for each odorant in the AEDA is equal to the highest dilution in which the odorant can be perceived at the sniffing port by three assessors.

The odour detection thresholds in the mimic matrix described before were calculated as previously reported (Pino & Queris, 2010). Calculation was made from the linear regression of percentage detection against log concentration. The 95% confidence limit calculated for the threshold values was used as a measure of error.

Quantitative descriptive aroma analysis was applied for evaluation of the wine, using a 10-cm unstructured scale anchored at its left and right extremes by the terms 'none' (0) and 'extremely strong' (9), respectively. The sensory evaluations were generated by a panel of nine trained assessors aged between 22 and 35 years. In the first session, panellists generated descriptive terms for the wine; in the second and third, different aroma standards were presented and discussed by panellists. From these discussions, seven aroma terms (fruity, sweet, winey, fermentation, caramel, flowery, and vinegar) were selected for further descriptive analysis. In the fourth and fifth sessions, the wine was evaluated in duplicate using the 10-point interval scale mentioned above. The reference materials for aroma descriptors were as follow: fruity (7 μg l^{-1} aqueous solution of ethyl 2-methylbutanoate), sweet (1 ml liquid caramel in 100 ml 10% ethanol–water solution), winey (5 ml of sherry wine in 100 ml 10% ethanol–water solution), fermentation (0.5 g dry yeast in 100 ml sugar solution after overnight), caramel (1 mg ml^{-1} aqueous solution of maltol), flowery (1 mg l^{-1} aqueous solution of 2-phenylethanol), and vinegar (5 mg ml^{-1} aqueous solution of acetic acid). Orthonasal evaluations were performed in coded cylindrical glass vessels (7 cm × 3.5 cm) containing 20 ml of wine.

2. Results and discussion

Although some studies of wines made from tropical fruits reported the use of direct sampling SPME (Selli et al., 2004; Kafkas et al., 2006), headspace sampling was selected for this study to avoid interferences from nonvolatile matrix components and to increase fibre lifetime. As can be seen in Table 2, a total of 102 volatile compounds were identified in the acerola wine, in which esters were found to be the most abundant volatile constituents (38 compounds), as they accounted for the largest proportion of the total aroma. Also, 19 alcohols, 16 acids, 8 terpenes, 5 aldehydes, 5 ketones, 3 furans, and other 8 of different chemical nature were identified in the acerola wine.

Table 2. Volatiles identified in acerola wine

Compound	LRI_a[a]	LRI_p[a]	Identity[b]	Area %
Ethanol	537	932	A	2.0
2-Methylpropan-1-ol	625	1108	A	0.8
Acetic acid	645	1450	A	4.2
Butan-1-ol	669	1150	A	0.4
Pentan-2-one	688	983	A	0.6
1-Hydroxypropan-2-one	694	1300	B	0.1
Pentan-3-one	703	984	A	<0.1
Ethyl propanoate	717	925	A	0.3

Table 2 (continued)

Compound	LRI_a [a]	LRI_p [a]	Identity[b]	Area %
Propyl acetate	728	977	A	0.1
1,1-Diethoxyethane	726	889	A	0.1
3-Methylbutan-1-ol	741	1212	A	13.1
2-Methylbutan-1-ol	742	1210	A	3.6
Dimethyl disulfide	745	1066	A	<0.1
Methyl 2-methylbutanoate	772	1015	A	2.6
2-Methylpropanoic acid	785	1580	A	<0.1
2-Methylpropyl acetate	788	1022	A	0.5
Ethyl butanoate	805	1044	A	0.5
Ethyl pyruvate	807	1242	A	0.3
2,3-Butanediol	810	1543	A	1.7
Ethyl 2-hydroxypropanoate	815	1358	A	0.1
2-Furfural	836	1165	A	11.6
3-Methylbutanoic acid	838	1022	A	<0.1
Ethyl 2-methylbutanoate	850	1050	A	0.8
(*E*)-3-Hexen-1-ol	854	1369	A	<0.1
Ethyl 3-methylbutanoate	857	1056	A	0.9
(*Z*)-3-Hexen-1-ol	859	1391	A	0.3
1,1-Diethoxy-2-methylpropane	861	969	B	0.2
Hexan-1-ol	871	1360	A	0.8
3-Methylbutyl acetate	881	1195	A	1.3
2-Methylbutyl acetate	884	1112	A	0.4
Heptan-2-one	892	1170	A	<0.1
Ethyl pentanoate	901	1131	A	0.2
γ-Butyrolactone	918	1647	B	<0.1
α-Pinene	940	1032	A	<0.1
5-Methyl-2-furfural	962	1560	A	12.2
Ethyl 2-hydroxy-3-methylbutanoate	965	1427	B	0.7
Heptan-1-ol	967	1310	A	<0.1
β-Pinene	977	1116	A	<0.1
1-Octen-3-ol	982	1394	A	0.3
Octan-3-one	984	1244	A	0.1
Hexanoic acid	988	1850	A	0.2
Octan-3-ol	991	1399	A	0.1
Ethyl hexanoate	998	1229	A	4.2
Octanal	999	1280	A	0.5
Ethyl (*Z*)-3-hexenoate	1002	1292	B	0.2
Hexyl acetate	1009	1276	A	0.1
1,4-Cineole	1016	1169	A	0.1

Table 2 (continued)

Compound	LRI_a [a]	LRI_p [a]	Identity[b]	Area %
4-Methyl-5-vinylthiazole	1021	1512	B	<0.1
Limonene	1029	1178	A	<0.1
2-Ethylhexan-1-ol	1032	1380	A	0.6
Salicylaldehyde	1043	1663	A	<0.1
Ethyl 2-furoate	1047	1627	A	0.2
Ethyl 2-hydroxy-4-methylpentanoate	1060	1547	B	0.8
3-Methylbutyl lactate	1065	1572	A	<0.1
Octan-1-ol	1068	1553	A	0.1
Allyl hexanoate	1083	1371	A	0.1
Heptanoic acid	1085	1966	A	0.1
Nonan-2-one	1091	1441	A	0.6
Ethyl heptanoate	1098	1337	A	0.5
Nonan-2-ol	1100	1532	B	<0.1
Nonanal	1103	1385	A	0.2
2-Phenylethanol	1107	1873	A	3.8
Methyl octanoate	1127	1386	A	<0.1
2-Ethylhexanoic acid	1128	1969	A	<0.1
2-Methylpropyl hexanoate	1150	1351	A	<0.1
Nerol oxide	1158	1467	B	<0.1
Nonan-1-ol	1169	1668	A	0.1
Ethyl benzoate	1173	1644	A	0.1
Diethyl succinate	1179	1687	A	12.2
Octanoic acid	1183	2050	A	0.6
α-Terpineol	1189	1694	A	<0.1
Ethyl octanoate	1197	1442	A	5.8
Decanal	1204	1502	A	0.1
β-Cyclocitral	1220	–	C	<0.1
Ethyl 2-phenylacetate	1245	1782	A	0.1
3-Methylbutyl hexanoate	1254	1733	A	<0.1
2-Phenylethyl acetate	1259	1825	A	0.1
Nonanoic acid	1297	2168	A	0.3
Ethyl nonanoate	1320	1520	A	0.3
Methyl anthranilate	1337	2188	A	<0.1
Ethyl 3-phenylpropanoate	1353	1900	A	<0.1
Decanoic acid	1379	2279	A	0.3
Ethyl (*E*)-4-decenoate	1383	1680	A	0.1
Ethyl (*E*)-9-decenoate	1389	1712	A	0.5
Ethyl decanoate	1396	1642	A	1.9
Dodecanal	1410	1729	A	<0.1

Table 2 (continued)

Compound	LRI_a [a]	LRI_p [a]	Identity[b]	Area %
Ethyl anthranilate	1416	2232	A	0.1
(*E*)-Geranyl acetone	1455	1803	B	<0.1
Ethyl (*E*)-cinnamate	1466	2149	A	<0.1
Dodecanoic acid	1569	2514	A	0.5
Ethyl dodecanoate	1595	1838	A	<0.1
n-Hexadecane	1600	1600	A	<0.1
Tridecanoic acid	1660	1924	A	0.1
n-Heptadecane	1700	1700	A	<0.1
Tetradecanoic acid	1779	2656	A	1.0
Ethyl tetradecanoate	1796	2044	A	<0.1
Pentadecanoic acid	1868	2819	A	0.8
(*Z*)-9-Hexadecenoic acid	1950	2960	A	0.2
Hexadecanoic acid	1960	2900	A	0.7
Heptadecan-1-ol	1986	2482	A	0.1
(*Z*)-9-Octadecenoic acid	2141	3172	A	0.1
Octadecanoic acid	2200	3092	A	0.3

[a]: LRI_a and LRI_p: Experimental linear retention index on capillary columns DB-5ms and DB-Wax; [b] : The reliability of the identification proposal is indicated by the following: A: mass spectrum and RI agreed with standards; B: mass spectrum and RI agreed with database or literature; C: mass spectrum agreed with mass spectral database

Among the esters, diethyl succinate, ethyl octanoate, and ethyl hexanoate were the major components in the acerola wine. The detected volatile esters can originate from alcoholic fermentation by yeast (ETIEVANT, 1991).

The volatile compounds extracted by HS-SPME were evaluated using AEDA and OAV to find the most potent odorants. The results of the AEDA and OAV studies are given in Table 3, in which odour zones are arranged following their elution order from the nonpolar column. The AEDA yielded 20 odour regions with flavour dilution (FD) factors ≥32. A great variety of odour qualities, such as fruity, sweet, caramel, or flowery were detected, but no single odorant resembled the acerola wine aroma. Sniffing of the serial dilutions revealed the highest FD factors for methyl 2-methylbutanoate (fruity), 2-ethylhexan-1-ol (sweet, slightly flowery), allyl hexanoate (fruity, sweet), and ethyl octanoate (fruity).

Two of the compounds identified as potentially relevant by AEDA were found with OAVs <1 and therefore, they should not contribute to acerola wine aroma. The possibly key compound obtained with the odour activity approach is a refinement of that provided by the AEDA, and corrects some of the limits of the AEDA technique.

Odour activity values are a good means to correlate quantitative data with the volatility of a compound from the respective matrix (SCHIEBERLE, 1995). However, it is necessary that the odour threshold of the compound should be determined in a matrix as close as possible to the food itself. For this reason, the odour thresholds for the components with higher DF were determined in a mimic matrix, representing the acerola wine (Table 3). By far, the highest values were calculated for methyl 2-methylbutanoate and 2-ethylhexan-1-ol. However, the results suggested that 18 compounds should additionally contribute to the characteristic

aroma of acerola wine, because their concentrations clearly exceeded their odour thresholds in water/ethanol. Relatively high odour activity values were also calculated for ethyl octanoate, ethyl hexanoate, ethyl 3-methylbutanoate, 5-methyl-2-furfural, and nonanal with OAVs ranged 9–101. With odour activity values between 1 and 4, ethanol, ethyl propanoate, 3-methylbutan-1-ol, ethyl pyruvate, 2,3-butanediol, 3-methylbutyl acetate, hexyl acetate, 2-phenylethanol, and ethyl 3-phenylpropanoate should also contribute to acerola wine aroma.

Table 3. Most odour-active volatile compounds identified in acerola wine

Compound	Content (mg l^{-1})	Odour quality[a]	Odour threshold (µg l^{-1})	FD factor	OAV[b]
Ethanol	114 000	alcohol	24 900[c]	–	5
Ethyl propanoate	0.017	rum-like, pineapple	10	64	2
3-Methylbutan-1-ol	0.700	fermented, fruity	280	64	2
2-Methylbutan-1-ol	0.190	fermented, fruity	300	64	<1
Methyl 2-methylbutanoate	0.141	fruity	0.5	512	281
Ethyl butanoate	0.028	fruity, sweet	20	32	1
Ethyl pyruvate	0.017	caramel	5	64	3
2,3-Butanediol	0.091	fruity	30	64	3
Ethyl 2-methylbutanoate	0.042	fruity, green	18	64	2
Ethyl 3-methylbutanoate	0.046	fruity, berry-like	3	128	15
3-Methylbutyl acetate	0.070	fruity, banana	30	32	2
5-Methyl-2-furfural	0.650	caramel-like	60	128	11
Ethyl hexanoate	0.225	fruity, winey	14	128	16
Hexyl acetate	0.005	fruity	2	64	2
2-Ethylhexan-1-ol	0.030	sweet, slightly flowery	0.3	512	101
Allyl hexanoate	0.005	fruity, sweet	0.1	256	47
Nonanal	0.009	orange-like	1	32	9
Diethyl succinate	0.310	winey	1250	32	<1
2-Phenylethanol	0.200	flowery, sweet	140	32	1
Ethyl octanoate	0.310	fruity	5	256	62
Ethyl 3-phenylpropanoate	0.001	flowery	0.65	64	2

[a]: Odour quality perceived at the sniffing port; [b]: Odour-activity values were calculated by dividing the concentrations by the respective odour threshold; [c]: The odour activity value for ethanol was calculated by dividing its concentration by its odour threshold in water.

Concerning similarities between HS-SPME-GC-O and OAV strategies, HS-SPME-GC-O has been very effective, since with a very small effort, it has been able to identify the most important odorants, according to the OAV criteria. Only ethanol ranked high according to OAV did not have a high GC-O score.

Figure 1 shows the mean intensity ratings for acerola wine plotted on a spider graph using six descriptors. In this diagram, the centre of the figure represents low intensity with respect to each descriptor, increasing to an intensity of nine at the ends of the axes. As can be seen, the fruity, sweet, flowery, and caramel series were those that contributed most markedly to the aroma profile. The fruity series was probably influenced mainly by allyl hexanoate, methyl 2-methylbutanoate, ethyl octanoate, and ethyl hexanoate. The sweet series had 2-ethylhexan-1-ol and allyl hexanoate as its main contributors, followed by ethyl butanoate and 2-phenylethanol. In the flowery series, the aroma contribution of ethyl 3-phenylpropanoate and 2-phenylethanol must also be the highest. The caramel series had 5-methyl-2-furfural and ethyl pyruvate as its main contributors. Other series with lesser impact were winey, fermentation, and vinegar.

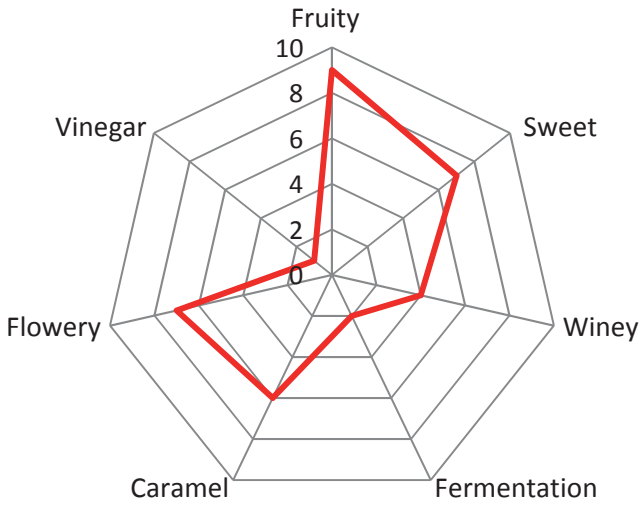

Fig. 1. Aroma sensory profiles of acerola wine

Sensory studies need to be done to determine the definite contribution of these volatile compounds to acerola wine, including model and omission experiments.

3. Conclusions

The study has revealed potent odorants that are responsible for the overall flavour of the acerola wine. Results of the AEDA and OAVs studies showed that odour profile of acerola wine was mainly caused by nineteen odorants, from which methyl 2-methylbutanoate and 2-ethylhexan-1-ol were the most odour-active compounds. These compounds are suitable indicators of the objective quality of the acerola wine. The high concordance of results between the OAV approach and HS-SPME-GC-O suggests that the latter has a great potential as a fast and simple tool to control and assess aroma quality of acerola wine.

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