ELSEVIER

Food Chemistry 74 (2001) 209–216

Food Chemistry

www.elsevier.com/locate/foodchem

Identification of the aroma components of acerola (*Malphigia glabra* L.): free and bound flavour compounds

R. Boulanger, J. Crouzet *

Université de Montpellier II, Laboratoire de Génie Biologique et Sciences des Aliments, Unité de Microbiologie et de Biochimie Industrielles Associée à l'INRA, 34095 Montpellier Cedex 05, France

Received 17 October 2000; accepted 2 January 2001

Abstract

Free and glycosidically-bound flavour compounds of acerola fruit were isolated and identified by GC and GC–MS analysis. Among the 46 compounds identified in the volatile fraction, the alcohols (3-methyl-but-3-en-1-ol, 3-methyl-butan-1-ol and 2-methyl-butan-1-ol) were predominant. Two other classes, aromatic compounds and esters, can participate in the fruity and fresh aroma of acerola. Among the 42 aglycones identified for the first time in this fruit, aliphatic alcohols and norisoprenoids were the main components. The latter were present in free form and only in traces. The hydrolysis of these aglycones could increase the fruity aroma of acerola. Four glucosides and one rutinoside were charaterized by GC–EIMS of their TFA derivatives.

Keywords: Acerola; *Malpighia glabra* or *emarginata*; Aroma; Volatile compounds; Aglycones; Glycosides

1. Introduction

The acerola, *Malpighia glabra* or *Malpighia emarginata*, is a shrub native to the West Indies. It also grows in Central and South America, Florida, Texas and Vietnam and it forms part of the family of Malpighiaceae. According to the country of origin, the fruit is known under various names, e.g. Acerola, West Indian cherry and Barbados cherry (Gomez, Reynes, Dornier, & Hebert, 1999). It is a small trilobate red fruit and quite similar to a cherry. The pulp is very juicy and cooling and possesses a fruity and sweet flavour but the fruit is principally known for its amount of vitamin C (Gomez et al., 1999; Maciel, Melo, De Lima, Da Silva, & Da Silva, 1999), varying between 1000 and 4500 mg/100 g of pulp, one of the most important natural sources. Thus, acerola is essentially employed to fortify fruit juice or to produce pharmacological and nutritional products. However, only few recent works have been focussed on this fruit. Herrmann (1981) studied the chemical composition. Chan and Yamamoto (1994) determined the kinetics of anthocyanin decomposition in acerola juice. Caceres, Lopez, Juarez, Del Aguila, and Garcia (1993) evaluated its antifungal activity for the treatment of dermatophytic infections and Ciolino (1998) worked on the compounds responsible for added caramel colour in adulterated acerola juice.

To the best of our knowledge, only one report (Schippa, George, & Fellous, 1993) exists on the volatile aroma compounds. Among the identified compounds, the presence of 3-methyl-but-3-en-1-ol, the major compound, and its esters, some of them identified for the first time, seem to participate in the fruity aroma of the pulp. Possibly another class of compounds, the norisoprenoids (C13), are responsible for part of the fruity aroma of acerola.

The potential aroma, constituted by glycosidically-bound aroma compounds, has been reported in many fruits, including tropical fruits. These compounds can be released during industrial pretreatment or processing of fruits, and generate modifications or a strengthening of the aroma (Kobayashi, Kubota, & Wang, 2000).

The objective of this work was, on the one hand, to identify the free volatile compounds recovered by two extraction methods, solid phase extraction and simultaneous distillation extraction and, on the other hand, to

* Corresponding author. Fax: +33-467-14-42-92.
E-mail address: crouzet@arpb.univ-montp2.fr (J. Crouzet).

0308-8146/01/$ - see front matter
PII: S0308-8146(01)00128-5

characterize the glycosidically-bound aroma compounds of acerola.

2. Materials and methods

2.1. Reagents

The solvents (*n*-pentane, dichloromethane and methanol) were pure grade (purity >97.7%) from Carlo Erba (Rodano, Italy) and were redistilled before use. Standards of *n*-Paraffin (C_8–C_{32}), purity >95.5%, were purchased from Sigma (St Quentin Fallavier, France). Amberlite XAD-2 (20–60 mesh), obtained from Röhm and Hass (Philadelphia, PA, USA), was treated according to the procedure of Günata, Bayonove, Baumes and Cordonnier (1985). Hemicellulase REG-2 (Gist-Brocades, Seclin, France) and sweet almond glucosidase — emulsin — (Sigma) were used. Trifluoroacetylating (TFA) reagent [*N*-methyl-bis (trifluoroacetamide)] was obtained from Pierce (Rockford, IL, USA). Reference compounds were obtained from commercial firms or received as a gift.

2.2. Plant material

Acerola at mature stage were purchased at Belém market and transported to France by plane. After washing, the fruit was crushed in a Waring blender for 3 min, the pulp was filtered through gauze to remove the seeds and centrifuged (30 min, 10 000×*g*) at 4°C; the clear juice obtained was kept at −18°C until analysis.

2.3. Simultaneous distillation extraction (SDE)

The extraction method was previously described by Boulanger and Crouzet (2000a). In a sample flask, 75 ml of clear juice of acerola were diluted with 250 ml of 0.2 M phosphate buffer, pH 7. Ten microlitres of nonan-4-ol (3.2 g/l in absolute ethanol) used as internal standard was added. The organic extract was dried over anhydrous sodium sulphate and concentrated to a final volume of 300 μl by microdistillation at 40°C (Bemelmans, 1979).

2.4. Solid phase extraction

Clear juice (50 ml) was poured onto a 9×1 cm i.d. column filled with solvent-washed XAD-2 at 1.5 ml/min and the separation of volatile compounds and glycosides was performed as indicated in Fig. 1 (Boulanger, Chassagne, & Crouzet, 1999).

2.5. Glycosyl glucose determination

Glycosyl glucose was determined using a HK/G-6-PDH spectrometric assay after acid hydrolysis of the crude glycosidically-bound fraction by sulfuric acid (2.25 M) during 1 h at 100°C (Williams, Cynkar, Francis, Gray, Iland & Coombe, 1995).

2.6. Enzymatic hydrolysis

The glycosidic extracts obtained from 45 ml of clear juice were concentrated in vacuum to dryness, redissolved in 0.3 ml of 0.2 M citrate-phosphate buffer (pH 5) and extracted five times using pentane-dichloromethane (2:1) to remove any traces of free compounds. Then, a mixture of hemicellulase REG-2 and sweet almond glucosidase was used to hydrolyse this extract at pH 5 (Boulanger et al., 1999).

2.7. Trifluoroacetylation

An aliquot of the methanolic solution obtained after elution of the XAD-2 column, corresponding to 2.5 ml of clear juice, was concentrated to dryness in a screw-capped vial at 60°C under a stream of nitrogen. Anhydrous pyridine (20 μl) and 20 μl of trifluoroacetylating (TFA) reagent were added and the vial tightly closed, stirred, heated at 60°C for 20 min, and then allowed to

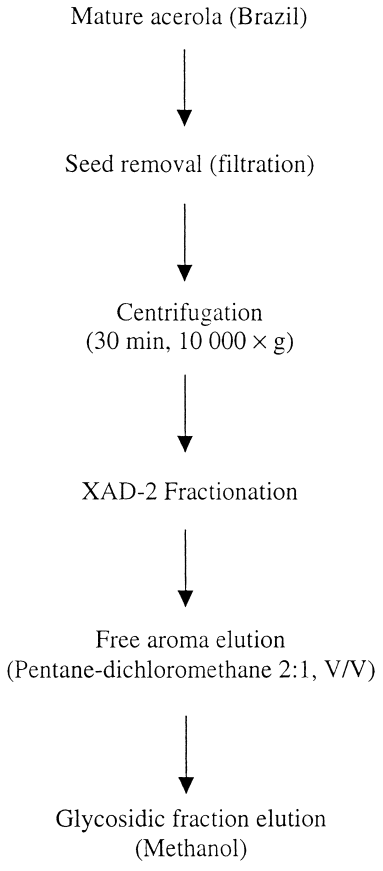

Fig. 1. Scheme of the solid phase extraction on XAD-2 of the acerola clear juice used to separate the volatile compounds and the glycosides.

cool to room temperature (Sweeley, Bentley, Makita, & Wells, 1963).

2.8. Volatile compounds and aglycons analysis

Capillary GC analysis was carried out on a Varian 3300 (Walnut Creek, CA, USA) gas chromatograph equipped with split injector (1/10) and flame ionization detector. The separation was achieved on two fused silica capillary columns (J & W Scientific, Folsom, CA, USA): (1) DB-WAX; and (2) DB-5MS (30 m×0.25 mm i.d., film thickness, 0.25 μm). The oven temperatures were programmed, respectively: (1) 3 min isothermal at 60°C and then increased at 2°C/min to 220°C, then from 220 to 250°C at 5°C/min and maintained for 15 min; and (2) 5 min isothermal at 40°C, increased from 40 to 200°C at 2°C/min, then from 200 to 250°C at 5°C/min and maintained for 15 min. In both cases the carrier gas was H_2 at 1.8 ml/min and N_2 at 30 ml/min was used for the make-up. The injector temperature was maintained at 250°C and the detector temperature was (1) 250°C, and (2) 300°C. Linear retention indices were calculated using *n*-paraffin standards (Van den dool & Kratz, 1963).

2.9. GC–MS analysis

Electronic impact mass spectrometric data were collected on an Automass 020 (Unicam, Argenteuil, France) mass spectrometer interfaced to a Varian 3400 gas chromatograph equipped with a DB-5MS fused silica capillary column (30 m×0.25 mm i.d.; 0.25 μm bonded phase, J & W Scientific). Injections were of 1 μl with a split ratio 1/10. The transfer line and the injector temperature were maintained at 250°C. The chromatographic conditions were the same as described for GC analysis. Helium at 2 ml/mn was the carrier gas. Source temperature was 150°C, mass spectra were scanned at 70 eV in the m/z range 40–250 at 1.0 s/decade.

2.10. TFA glycosides analysis

The chromatographic conditions of this analysis were previously described by Boulanger et al. (1999).

2.11. Identification and quantification

Identifications of the free and glycosidically-bound compounds were based on the comparison of linear retention indices and mass spectral data with reference compounds, with literature data or with data bank (Wiley Mass Spectral Data). Three analyses, extractions and measurements, were carried out on each extract. Nonan-4-ol was used as internal standard for quantification. It was assumed that the response factor was equal to one for all the compounds. The standard deviation was determined for each component identified.

3. Results and discussion

3.1. Free volatile compounds

The volatile fraction was extracted by two methods in order to take into consideration the generation of artefact compounds. The free aroma compounds, separated on two columns of different polarity (DB5-MS and DB-WAX), are listed in Table 1. The total quantities extracted by the two methods, 19 mg/kg of pulp by the solid phase extraction and 33 mg/kg of pulp after SDE, were quite different. Among the 46 compounds identified or tentatively identified, 16 are reported for the first time in this fruit. The volatile fraction was constituted of nine alcohols, 21 esters, seven aromatic compounds, two terpenic compounds, two norisoprenoids, three acids and two lactones.

As indicated in Table. 1, the most abundant compounds were the aliphatic alcohols. Among them, 3-methyl-but-3-en-1-ol, 3-methyl-butan-1-ol and 2-methyl-butan-1-ol were predominant. The first compound was previously detected by Schippa et al. (1993) as the main component in acerola. It was also identified in pepino fruit by Shiota, Young, Paterson, and Irie (1988). Moreover, six derived esters of this alcohol were also detected by Schippa et al. (1993). These authors propose that these alcohols and esters contribute to the typical fruity note of acerola. The catabolism of amino acids is at the origin of 3- and 2-methyl-butan-1-ol and their esters. According to Tressl and Albrecht (1986), the enzymatic degradation of leucine led to the first one of these and its esters, whereas the work of Wyllie, Leach, Nonhebel, and Lunsunzi (1996), has shown that isoleucine gives the 2-methyl-butan-1-ol and derived compounds. The 3-methyl-but-3-en-1-ol and its esters could arise from this enzymatic mechanism. Moreover, the diphosphate ester of this alcohol is known as a key intermediate in terpene biosynthesis; the small quantities of terpenic compounds and the great quantity of 3-methyl-but-3-en-1-ol detected in acerola could be due to the fact that this terpenic pathway is not effective in acerola. The difference of individual content of alcohols between the two extractions could be explained by different hypotheses: on the one hand, a retention of compounds during juice clarification and, on the other hand, by rearrangement reactions and partial hydrolysis during the SDE extraction method.

Qualitatively, the most abundant compounds were esters; nine compounds are reported for the first time and seven can be derived from the four major alcohols. As observed in the volatile fraction of cupuaçu, the total quantity of esters was quite similar in the two extraction methods although some differences were noticed for several compounds. This class of compounds must participate in the fruity and sweet acerola aroma as in other tropical fruits, such as cupuaçu (Fischer, Hammerschmidt, & Brunke, 1995), pepino fruit (Shiota et al., 1988),

Table 1
Aroma compounds identified or tentatively identified in acerola (*Malpighia glabra* L.) pulp after Amberlite XAD-2 resin and SDE extraction

Compound	RI[a]	RI[b]	Concentration (μg/kg of pulp)	
			XAD-2 extract (±S.D.)	SDE extract
Alcohols				
3-methyl-but-3-en-1-ol	720	1226	2976±750	12354±100
3-methyl-butan-1-ol	726	1213	5515±838	9261±50
2-methyl-butan-1-ol[c]	730	1217	1652±237	2626±20
3-methyl-but-2-en-1-ol[c]	770	1324	305±68	1484±49
Furfuryl alcohol	845	1620	nd[f]	72±1
(*Z*)-hex-3-en-1-ol	846	1387	619±42	857±2
Hexan-1-ol	863	1359	977±78	1145±6
Oct-1-en-3-ol	963	1428	164±10	181±1
Octan-3-ol	971	1396	tr[e]	73±1
Aromatic compounds				
Toluene[c]	–	1034	18±2	443±29
Benzyl alcohol	1025	1861	317±130	56±2
2-phenyl ethanal	1037	1618	nd	40±5
2-phenyl ethanol	1103	1889	2686±12	1264±22
3-phenyl-propan-1-ol[c,d]	1219	–	31±5	nd
4-ethyl-guaiacol[c]	1268	1987	nd	tr
4-vinyl-guaiacol	1301	2176	nd	143±1
Esters				
Ethyl butanoate	798	1043	703±161	708±16
Butyl acetate	810	–	30±1	130±3
Methyl 3-methyl-3-hydroxy-butanoate[d]	871	–	nd	tr
3-methyl-but-1-yl acetate[d]	872	–	568±45	614±5
3-methyl-but-3-en-1-yl acetate[d]	879	–	667±51	672±4
3-methyl-but-2-en-1-yl acetate[c,d]	902	–	41±1	90±1
Ethyl 3-methyl-3-hydroxy-butanoate[c,d]	920	–	99±5	184±1
Ethyl hexanoate	989	1212	158±3	224±3
Hexyl acetate[c]	1043	1276	tr	nd
Ethyl 2-furoate[d]	1047	–	106±3	94±1
3-methyl-but-3-en-1-yl butanoate[d]	1063	–	22±3	tr
Diethyl propanedioate[c,d]	1069	–	115±9	tr
Ethyl 3-hydroxy-hexanoate[d]	1125	–	24±5	65±8
Ethyl benzoate	1160	1672	tr	39±5
Diethyl butanedioate[c,d]	1165	–	tr	tr
3-methyl-but-3-en-1-yl 3-methyl-but-2-enoate[d]	1176	–	107±4	70±1
Ethyl octanoate	1191	1435	89±3	54±1
Ethyl phenylacetate[c]	1229	1826	tr	tr
2-phenylethyl acetate[c]	1249	1782	88±5	83±1
Diethyl pentanedioate[c,d]	1281	–	96±3	90±2
Ethyl nonanoate[c]	1296	1553	19±2	nd
Terpenic compounds				
Limonène	1017	1213	193±30	nd
Linalol[c]	1098	1556	112±11	106±1
Norisoprenoids				
2,5-epoxymegastigma-6(*E*),8(*E*)-diene[d]	1320	–	nd	tr
3-oxo-α-ionol	1632	2615	tr	nd
Acids				
Butanoic acid	790	1599	tr	nd
Hexanoic acid	989	1836	tr	nd
Heptanoic acid	1180	1915	108±77	nd
Lactones				
γ-hexalactone	1036	1679	201±16	98±1
δ-nonalactone[c]	1270	2138	44±10	nd

[a] RI, Linear retention index on DB-5MS.
[b] RI, Linear retention index on DB-WAX.
[c] Compound newly identified.
[d] Compound tentatively identified.
[e] tr, < 5 μg/kg.
[f] nd, not detected.

(Table continued on next page)

mango (Adedeji, Hartman, Lech, & Ho, 1992), soursop (Wong & Khoo, 1993) guava (MacLeod & De Troconis, 1982), cashew apple (Bicalho, Pereira, Aquino Neto, Pinto, & Rezende, 2000) and pineapple (Umano, Hagi, Nakahara, Shoji, & Shibamoto, 1992).

2-Phenylethanol represented more than 80% of aromatic compounds. It can be assumed that this compound, present at concentrations varying between 2686 and 1264 μg/kg of pulp, according to the extraction procedure, and having a low threshold (86 ppb in water), can participate at the floral aroma of acerola. As in cupuaçu (Boulanger & Crouzet, 2000a); the other aromatic compounds were only minor compounds.

Unlike other Amazonian fruits, cupuaçu and bacuri studied by Boulanger et al. (1999), or camu-camu, umbu-caja and araca-boi (Franco & Shibamato, 2000), the terpenic compounds were present in a very weak quantities, as previously stated. Only linalol and limonene were detected.

The other compounds identified, such as acids, lactones and norisoprenoids, were also present in small quantities (Table. 1), except for the γ-hexalactone previously identified by Schippa et al. (1993). Whereas these authors have characterized eight norisoprenoids in the volatile fraction, we only detected the 3-oxo-α-ionol and the 2,5-epoxymegastigm-6(*E*),8(*E*)-diene. Nevertheless, these compounds were detected only in the bound fraction, as in grape (Razungles, Günata, Pinatel, Baumes, & Bayonove, 1993; Pabst, Barron, Etievant, & Schreier, 1991) and passion fruit (Winterhalter, 1990), so it can be assumed that the presence of norisoprenoids in the volatile fraction reported by Schippa et al. (1993) may be the result of the hydrolysis of glycosides during the extraction process performed at the natural pH of fruit.

3.2. Glycosidically bound compounds

In order to know the importance of the glycosidically-bound fraction, we determined the proportion of glycosyl glucose by the method described by Williams et al. (1995). Nevertheless, this technique may give overestimated results if the glycosidic moiety of some glycosides comprises several glucose units, as in gentiobiosides. For acerola, the result was 1483 ± 140 μmol glucose/kg of pulp. It was more than bacuri and purple passion fruit (Boulanger et al., 1999); they have, respectively, 1387 ± 180 and 1250 ± 100 μmol glucose/kg of pulp. The latter is known to have an important glycosidic fraction (Chassagne, Crouzet, Bayonove, & Baumes, 1998).

According to Günata, Bitteur, Brillouet, Bayonove, and Cordonnier (1988), the complete hydrolysis of glycoside needs several enzymatic activities, such as arabinopyranase, rhamnopyranase and glucosidase, to allow the sequential enzymatic mechanism. Hemicellulase REG2 and sweet almond glucosidase possess these different activities (Cordonnier, Günata, Baumes, & Bayonove, 1989). According to the previously reported data on passion fruit (Chassagne, Boulanger, & Crouzet, 1999) and on Amazonian fruits (Boulanger & Crouzet, 2000b), a mixture of these two preparations seemed sufficient to hydrolyse the acerola glycosides.

The volatile compounds released by enzymatic treatment of the glycosidic fraction are given in Table 2. Among the 42 aglycones identified for the first time in the acerola pulp, 17 were detected in free form. This fraction was characterized by 13 norisoprenoid compounds, 11 aromatic components, eight aliphatic alcohols, four hydroxy-esters, three terpenic compounds, two lactones and one acid.

As in the volatile fraction, the alcohols were quantitatively the most important compounds and 3-methylbut-3-en-1ol was the most abundant. The detection of this molecule in bound form is in agreement with the fact that this compound is a key intermediate in various biosynthetic pathways. The liberation of alcohols can reinforce the fruity and green aroma of acerola. The presence of aliphatic alcohol glycosides was previously detected in apple (Schwab, Scheller, Gerlach, & Schreier, 1989; Schwab & Schreier, 1990) and passion fruit (Chassagne, Crouzet, Bayonove, Brillouet, & Baumes, 1996).

As previously stated, norisoprenoids were principally found in the glycosidic fraction and were qualitatively the most important class of compounds; indeed the norisoprenoid compounds are very odorous. Among them, 3-oxo-α-ionol, 4-oxo-β-ionol, 4-hydroxy-β-ionol and an epoxy-megastigmadiene, previously detected by Schippa et al. (1993) in the volatile fraction of acerola, were predominant. This class was also detected in the glycosidically-bound fraction of several fruits: grape (Strauss, Wilson, & Williams, 1987; Sefton, Skouroumounis, Massy-Westropp, & Williams, 1989), quince (Winterhalter & Schreier, 1988a; Winterhalter, Herderich, & Schreier, 1990), apple (Schwab & Schreier, 1990), raspberry (Pabst et al., 1991) and passion fruit (Chassagne et al., 1999). The liberation of norisoprenoids can intensify the fruity note of acerola either directly or indirectly. In fact, these aglycones can be transformed in other aroma compounds in acid conditions (Winterhalter & Schreier, 1988b). According to Winterhalter and Schreier (1988c), 4-hydroxy-7,8-dihydro-β-ionol was a natural precursor of theaspirane.

The other aglycones were present in small quantities except 2-phenylethanol, benzyl alcohol, ethyl 3-methyl-3-hydroxy-butanoate and hexanoic acid. These compounds were also identified in the volatile fraction; however their liberation may play a smaller role in the acerola aroma than the aliphatic alcohols and the norisoprenoids.

According to Voirin, Baumes, Sapis, and Bayonove (1992), glycoside structures, after trifluoracetylation,

Table 2
Aglycones released by enzymatic hydrolysis from acerola crude heterosidic extract

Compound	RI[a]	RI[b]	Concentration (μg/kg of pulp; ±S.D.)
Aliphatic alcohols			
3-methyl-but-3-en-1-ol[c]	720	1230	384±39
3-methyl-butan-1-ol[c]	726	1212	71±9
2-methyl-butan-1-ol[c]	730	1216	42±7
(*Z*)-hex-3-en-1-ol[c]	848	1390	19±4
Hexan-1-ol[c]	872	1359	171±23
Oct-1-en-3-ol[c]	965	1420	37±3
Octan-3-ol[c]	980	1392	28±4
Octan-1-ol	1070	1563	60±4
Derived norisoprenoids			
Theaspirane A[d]	1280	–	tr[e]
Theaspirane B[d]	1299	–	tr
2,5-epoxy-megastigma-6(*Z*),8(*E*)-diene[d]	1312	–	tr
2,5-epoxy-megastigma-6(*E*),8(*E*)-diene[c,d]	1324	–	104±8
3,4-dihydro-γ-ionol[d]	1371	–	10±1
β-damascenone	1387	1819	24±4
4-hydroxy-β-ionol[d]	1600	–	154±10
2-hydroxy-β-ionone[d]	1619	–	58±7
3-hydroxy-β-ionol[d]	1622	–	32±6
3-oxo-α-ionol[c]	1629	2623	175±20
4-oxo-β-ionol	1660	2630	141±20
3-hydroxy-β-ionone[d]	1676	–	49±5
3-oxo-retro-α-ionol	1698	2721	18±3
Aromatic compounds			
benzyl alcohol[c]	1029	1856	48±8
4-methoxy-phenol[d]	1071	–	tr
2-phenyl ethanol[c]	1103	1894	97±17
4-ethyl phenol	1161	2117	tr
3-phenyl-1-propanol[c,d]	1221	–	tr
2-methyl benzaldehyde[d]	1222	–	tr
4-ethyl guaiacol[c]	1260	1983	10±1
Eugenol	1341	2143	tr
Vanillin	1382	2453	tr
4-allyl-2,6-dimethyl-phenol[d]	1680	–	tr
coniferylic alcohol[d]	1726	–	29±3
Terpenics compounds			
Trans linalol furanoxide	1081	1458	19±4
Linalol[c]	1094	1551	15±1
(*Z*)-2,6-dimethyl-octa-2,7-dien-1,6-diol	1361	2327	13±1
Esters			
Ethyl 3-hydroxy-butanoate[d]	913	–	tr
Ethyl 3-methyl 3-hydroxy-butanoate[c,d]	926	–	58±5
Methyl 3-hydroxy-hexanoate	1049	1640	13±3
Ethyl 3-hydroxy-pentanoate[d]	1129	–	12±1
Acids			
Hexanoic acid[c]	977	1832	187±3
Miscellaneous compounds			
γ-hexalactone[c]	1038	1673	26±3
δ-octalactone	1268	1891	tr

[a] RI, Linear retention index on DB-5MS.
[b] RI, Linear retention index on DB-WAX.
[c] Compound previously identified in the volatile fraction.
[d] Compound tentatively identified.
[e] tr, <5 μg/kg.

Table 3
Electronic impact mass spectrometric data of glycosides identified as TFA derivatives in acerola

Glycoside	RRT[a]	RRT[b]	EI mass spectrometric data	
			Aglycone moiety	Saccharidic moiety
Monoglucosides (β-D-glucopyranoside)				
Hexan-1-ol	0.86	0.85	85(100), 57(40), 69(18)	193(5), 319(4), 177(2)
Benzyl alcohol	1.16	1.17	91(100), 92(19), 69(10), 108(3), 107(2)	193(5), 319(1)
2-Phenylethanol	1.44	1.45	105(100), 104(43), 69(42), 91(32), 106(10)	319(5), 193(2), 177(1)
3-Oxo-α-ionol	2.57	–	108(100), 91(43), 69(30), 135(13), 97(3), 81(3)	319(19), 193(3)
Rutinoside (α-L-rhamnopyranosyl-β-D-glucopyranoside)				
Benzyl alcohol	1.99	2.03	91(100), 92(12), 107(5), 69(5), 108(1)	193(19), 207(4)

[a] RRT, relative retention time of natural compound compared with the internal standard (phenyl-glucoside).
[b] RRT, relative retention time of reference compound compared with the internal standard.

can be tentatively established by direct GC/EI–MS. Acerola glycosides were identified using their mass spectra and comparison of their retention times with those of authentic reference samples, except for the 3-oxo-α-ionyl glucoside. Among the TFA glycosides separated by GC, only four glucosides and one rutinoside were characterized (Table 3). Despite the proportion of aliphatic alcohols and derived norisoprenoids, only one glucoside of each class was identified, the hexyl and 3-oxo-α-ionyl glucosides. The last was detected by the study of its mass spectrum, which has characteristic fragment ions for this aglycone (m/z 108 and 91). This glucoside was previously reported in strawberry (Pabst, Barron, Semon, & Schreier, 1992), grape (Baumes, Aubert, Günata, De Moor, Bayonove, & Tapiero, 1994) and passion fruit (Chassagne et al., 1996). Though the 3-methyl-but-3-en-1-ol was the most abundant aglycone, no glycoside of this compound was identified.

4. Conclusions

Among the free aroma components, three classes of compounds were predominant, aliphatic alcohols, esters and aromatic compounds. The glycosidically-bound fraction was composed of aliphatic alcohols, as observed in the volatile fraction, and norisoprenoids. However, after acid hydrolysis of this fraction, it seemed that the glycone moiety could contain unusual glycosides. Their presence can be inferred because only five glycosides have been characterized by TFA glycoside analysis. Therefore, extraction and purification of glycosides will provide further information about the glycone structures of acerola compounds.

Acknowledgements

This work was supported by the European Union, contract STD ERB-TS3-CT94-0300, and one of the authors (R.B.) received financial support from the Ministère de l'Education Nationale de la Recherche et de la Technologie. The authors are indebted to Pr. P. Winterhalter, Der Technischen Universität Carolo-Wilhelmina, Braunschweig, and Dr. R. Baumes, Institut des Produits de la Vigne, INRA, Montpellier for the gift of reference compounds.

References

Adedeji, J., Hartman, T. G., Lech, J., & Ho, C. T. (1992). Characterization of glycosidically bound aroma compounds in the African mango (*Mangifera indica* L.). *Journal Agricultural and Food Chemistry, 40*, 659–661.

Baumes, R. L., Aubert, C. C., Günata, Z. Y., De Moor, W., Bayonove, C. L., & Tapiero, C. (1994). Structures of two C_{13} norisoprenoid glucosidic precursors of wine flavor. *Journal of Essential Oil Research, 6*, 587–599.

Bemelmans, J. M. (1979). Review of isolation and concentration technique. In D. G. Land, & H. E. Nursten, *Progress in flavour research* (pp. 79–98). London: Applied Science Publishers.

Bicalho, B., Pereira, A. S., Aquino Neto, F. R., Pinto, A. C., & Rezende, C. M. (2000). Application of high-temperature gas chromatography-mass spectrometry to the investigation of glycosidically bound components related to cashew apple (*Anacardium occidentale* L. Var. *nanum*) volatiles. *Journal Agricultural and Food Chemistry, 48*, 1167–1174.

Boulanger, R., Chassagne, D., & Crouzet, J. (1999). Free and bound flavour compounds of Amazonian fruits. 1: Bacuri. *Flavour and Fragrance Journal, 14*, 303–311.

Boulanger, R., & Crouzet, J. (2000a). Free and bound flavour of Amazonian fruits: 2. Cupuaçu volatile compounds. *Flavour and Fragrance Journal, 15*, 251–257.

Boulanger, R., & Crouzet, J. (2000b). Free and bound flavour of Amazonian fruits: 3. glycosidically volatile compounds of cupuaçu. *Food Chemistry, 70*, 463–470.

Caceres, A., Lopez, B., Juarez, X., Del Aguila, J., & Garcia, S. (1993). Plants used in Guatemala for the treatment of dermatophytic infections. 2. Evaluation of antifungal activity of seven American plants. *Journal of Ethnopharmacology, 40*, 207–213.

Chan, H. T., & Yamamoto, H. Y. (1994). Kinetics of anthocyanin decomposition in acerola juice. *ASEAN Food Journal, 9*, 132–135.

Chassagne, D., Boulanger, R., & Crouzet, J. (1999). Enzymatic hydrolysis of edible *Passiflora* fruit glycosides. *Food Chemistry, 66*, 281–288.

Chassagne, D., Crouzet, J., Bayonove, C., & Baumes, R. (1998). Identification of passion fruit glycosides by gas chromatography/mass spectrometry. *Journal Agricultural and Food Chemistry, 46*, 4352–4357.

Chassagne, D., Crouzet, J., Bayonove, C., Brillouet, J. M., & Baumes, R. (1996). 6-O-α-L-arabinopyranosyl-β-D-glucopyranosides as aroma precursors from passion fruit. *Phytochemistry, 41*, 1497–1500.

Cioliono, L. A. (1998). Determination and classification of added caramel color in adulterated acerola juice formulations. *Journal Agricultural and Food Chemistry, 46*, 1746–1753.

Cordonnier, R. E., Günata, Y. Z., Baumes, R. L., & Bayonove, C. L. (1989). Recherche d'un matériel enzymatique adapté à l'hydrolyse des précurseurs d'arôme de nature glycosidique du raisin. *Journal International des Sciences de la Vigne et du Vin, 23*, 7–23.

Fischer, N., Hammerschmidt, F. J., & Brunke, E. J. (1995). Analytical investigation of the flavor of cupuaçu (*Theobroma grandiflorum* Spreng.). In R. L. Rousseff, & M. M. Leahy, *Fruits flavors. Biogenesis, characterization, and authentification* (pp. 8–20). Washington, DC: American Chemical Society, Symposium Series.

Franco, M. R., & Shibamoto, T. (2000). Volatile composition of some Brazilian fruits: Umbu-caja (*Spondias citherea*), Camu-camu (*Myrciaria dubia*), Araca-boi (*Eugenia Stipitata*) and Cupuaçu (*Theobroma grandiflorum*). *Journal Agricultural and Food Chemistry, 48*, 1263–1265.

Gomez, P., Reynes, M., Dornier, M., & Hebert, J. P. (1999). La cerise des Antilles: une exceptionnelle source de vitamine C naturelle. *Fruits, 54*, 247–260.

Günata, Y. Z., Bayonove, C. L., Baumes, R. L., & Cordonnier, R. E. (1985). The aroma of grapes: I. extraction and determination of free and glycosidically bound fractions of some grape aroma components. *Journal of Chromatography, 331*, 83–90.

Günata, Z., Bitteur, S., Brillouet, J. M., Bayonove, C., & Cordonnier, R. (1988). Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research, 184*, 139–149.

Herrmann, K. (1981). Übersicht über die chemische Zusammensetzung und die Inhaltsstoffe einer reihe wichtiger exotischer Obstarten. *Zeitschrift für Lebensmittel-Untersuchung-Forschung, 173*, 47–60.

Kobayoshi, A., Kubota, K., & Wang, D. (2000). Qualitative and quantitative analyses of glycosides as aroma precursors during black tea manufacturing process. In P. Schieberle, & K.-H. Engel, *Frontiers of flavour science* (pp. 452–456). Garching: Deutsche Forschungsanstalt für Lebensmittelchemie.

Mac Leod, A. J., & De Troconis, N. G. (1982). Volatile flavour components of guava. *Phytochemistry, 21*, 1339–1342.

Maciel, M. I., Melo, E., De Lima, V., Da Silva, M., & Da Silva, I. (1999). Processing and storage of acerola (Malpighia sp) fruit and its products. *Journal of Food Science and Technology, 36*, 142–146.

Pabst, A., Barron, D., Etievant, P., & Schreier, P. (1991). Studies on the enzymatic hydrolysis of bound aroma constituents from raspberry fruit pulp. *Journal Agricultural and Food Chemistry, 39*, 173–175.

Pabst, A., Barron, D., Semon, E., & Schreier, P. (1992). Two diastereomeric 3-oxo-α-ionol β-D-glucosides from raspberry fruit. *Phytochemistry, 31*, 1649–1652.

Razungles, A., Günata, Z., Pinatel, S., Baumes, R., & Bayonove, C. (1993). Etude quantitative de composés terpèniques, norisoprénoides et de leurs précurseurs dans diverses variétés de raisins. *Sciences des Aliments, 13*, 59–72.

Schippa, C., George, G., & Fellous, R. (1993). Constituants volatils de l'acérola. *Parfums, Cosmétiques, Arômes, 113*, 81–84.

Schwab, W., Scheller, G., Gerlach, D., & Schreier, P. (1989). Identification of 3-hydroxyoctyl β-D-glucoside and absolute configuration of free and bound octane-1,3-diol in apple fruit. *Phytochemistry, 28*, 157–160.

Schwab, W., & Schreier, P. (1990). Glycosidic conjugates of aliphatic alcohols from apple fruit (*Malus sylvestris* Mill Cult. Jonathan). *Journal Agricultural and Food Chemistry, 38*, 757–763.

Sefton, M. A., Skouroumounis, G., Massy-Westropp, R., & Williams, P. (1989). Norisoprenoids in *Vitis vinifera* white wine grapes and the identification of a precursor of damascenone in these fruits. *Australian Journal of Chemistry, 42*, 2071–2084.

Shiota, H., Young, H., Paterson, V., & Irie, M. (1988). Volatile aroma constituents of pepino fruit. *Journal of the Science of Food and Agriculture, 43*, 343–354.

Strauss, C., Wilson, B., & Williams, P. (1987). 3-Oxo-α-ionol, vomifoliol and roseoside in *Vitis vinifera* fruit. *Phytochemistry, 26*, 1995–1997.

Sweeley, C., Bentley, R., Makita, H., & Wells, M. (1963). Gas liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *Journal of the American Chemical Society, 85*, 2497–2507.

Tressl, R., & Albrecht, W. (1986). Biogenesis of aroma compounds through acyl pathways. In T. H. Parliment, & R. Croteau, *Biogeneration of aromas* (pp. 114–133). Washington, DC: American Chemical Society, Symposium series.

Umano, K., Hagi, Y., Nakahara, K., Shoji, A., & Shibamoto, T. (1992). Volatile constituents of green and ripened pineapple (*Ananas comosus* [L.] Merr.). *Journal Agricultural and Food Chemistry, 40*, 599–603.

Van den dool, H., & Kratz, P. D. A. (1963). A generalization of the retention index system including linear temperature programmed gas liquid partition chromatography. *Journal of Chromatography, 11*, 463–473.

Voirin, S. G., Baumes, R. L., Sapis, J. C., & Bayonove, C. L. (1992). Analytical methods for monoterpene glycosides in grape and wine. II Qualitative and quantitative determination of monoterpene glycosides in grape. *Journal of Chromatography, 595*, 269–281.

Williams, P. J., Cynkar, W., Francis, I. L., Gray, J. D., Iland, P. G., & Coombe, B. G. (1995). Quantification of glycosides in grapes, juices, and wines though a determination of glycosyl glucose. *Journal Agricultural and Food Chemistry, 43*, 121–128.

Winterhalter, P. (1990). Bound terpenoids in the juice of the purple passion fruit (*Passiflora edulis* Sims). *Journal Agricultural and Food Chemistry, 38*, 452–455.

Winterhalter, P., Herderich, M., & Schreier, P. (1990). 4-Hydroxy-7,8-dihydro-β-ionone and isomeric megastigma-6,8-dien-4-ones: new C_{13} norisoprenoids in quince (*Cydonia oblonga*, Mill.) fruit. *Journal Agricultural and Food Chemistry, 38*, 796–799.

Winterhalter, P., & Schreier, P. (1988a). Free and bound C_{13} norisoprenoids in quince (*Cydonia oblonga*, Mill.) fruit. *Journal Agricultural and Food Chemistry, 36*, 1251–1256.

Winterhalter, P., & Schreier, P. (1988b). Studies on C_{13}-norisoprenoids precursors. In P. Schreier, *Bioflavour '87* (pp. 255–273). Berlin: De gruyter & Co.

Winterhalter, P., & Schreier, P. (1988c). 4-Hydroxy-7,8-dihydro-β-ionol: natural precursor of theaspiranes in quince fruit (*Cydonia oblonga*, Mill.). *Journal Agricultural and Food Chemistry, 38*, 560–562.

Wong, K. C., & Khoo, K. H. (1993). Volatile components of Malaysian *Annona* fruits. *Flavour and Fragance Journal, 8*, 5–10.

Wyllie, S. G., Leach, D. N., Nonhebel, H. N., & Lunsunzi, I. (1996). Biochemical pathways for the formation of esters in ripening fruit. In A. J. Taylor, & D. S. Mottram, *Flavour science* (pp. 52–57). Bodmin: The Royal Society of Chemistry.