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Chemical composition of acerola fruit (*Malpighia punicifolia* L.) at three stages of maturity

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Abstract

The effect of stage of maturity on the chemical composition and volatile components of acerola *(Malpighia punicifolia* L.*)* was investigated in three different stages. Stage of maturation was characterized by different indicators such as color, vitamin C, soluble solids, protein, ash, moisture, titrable acidity, pH and sugars. Titrable acidity, sugars and soluble solids increased and vitamin C and protein decreased with the progress of maturation. The volatile fraction was analysed by GC–MS. It was possible to identify 31 compounds in the mature (red) fruits, such as acethyl-methyl-carbinol, 2-methyl-propyl-acetate, limonene, E-Z-octenal, ethyl hexanoate, isoprenyl butirate and acetofenone; 23 in the intermediate (yellow), such as, methyl hexanoate, 3-octen-1-ol and hexyl butirate; and 14 in the immature (green) fruit, such as methyl-propyl-ketone, E-Z-hexenyl-acetate and 1-octadecanol. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Acerola; *Malpighia punicifolia* L.; Gas chromatography; Ripening; Flavor; Aroma

1. Introduction

Acerola (*Malpighia punicifolia* L.) is a plant originated in Central America that has been propagated to South America including Brazil due to its good adaptation to soil and climate. It is nutritionally important, mainly due to its very high level of vitamin C. The plant provides flowers and fruits at different stages and, consequently, long fruiting periods during the year are observed. The fruit presents a short living period after being picked (2 to 3 days), at room temperature. Ripening of the fruit involves a series of complex biochemical reactions such as hydrolysis of starch, conversion of chloroplasts into chromoplasts with chlorophyll transformation, production of carotenoids, anthocyanins and phenolics and the formation of volatile compounds. All these reactions are important for the final characteristics of the mature fruit and for its peculiar flavour (Speirs & Brady, 1991).

Many reports are found in the literature on the chemical composition of acerola fruit, however, they have concentrated mainly on the non-volatile fraction with emphasis on vitamin C (Alves, 1993; Asenjo, 1980; Asenjo & Muñiz, 1954; Leme, Fonseca & Nogueira, 1973, López, 1963; Miller, Wenkam & Fitting, 1961; Moscoso, 1956; Simão, 1971). Works related to the volatile fraction composition are scarce, despite their importance for the aroma of the fruit (Schippa, George & Fellows, 1993)

Chemical composition, including the distribution of aroma components, is dependent on the species, environmental conditions, and also on the stage of maturity of the fruit. In the present work, the composition of *Malpighia punicifolia* L. was studied at different stages of maturity. Non-volatile components, including ascorbic acid were evaluated. Complementary, the volatile fraction was also studied and relevant components were characterised.

2. Materials and methods

2.1. Fruit

Acerola (*M. punicifolia* L.) fruits were harvested from different trees in the region of Maricá, Rio de Janeiro

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State, Brazil, during the month of March. Fruits were picked up at three different stages of maturity based on the colour of the peel. The fruits were stored at −12°C prior to the analysis. For the present study, full green was chosen as the initial stage, yellow as the middle and red as the last stage of maturity.

2.2. Extraction

Samples (300 g of the fruit) were homogenised with 150 ml of water in a blender, passed through a sieve to separate the seeds and then extracted with 300 ml of diclorometh ane in an ultrasonic bath for 2 h as described by Schippa et al. (1993). The extract was filtered through anhydrous sodium sulphate and concentrated to 5 ml under vacuum and then to 0.5 ml under nitrogen flow.

2.3. Non-volatile analysis

The fruits at different stages of maturity were characterised by different indicators such as colour, soluble solids, vitamin C, titratable acidity, protein, ash, moisture, pH and sugars. The color was instrumentally determined by using a S&M Color Computer Suga SM-4-CH (USA). The readings were made on a double face basis of the fruit (internal and external) arranged on the transparent crystal (10 cm diameter × 1 cm; light $L=12.17$; $a=-1.63$; $b=-18.87$). The average of 10 determinations were then considered. From Hunter values of (a)= −80 to 0 = green and 0 to +100 = red; (b)= −100 to 0 = blue and 0 to +70 = yellow; (L) luminosity 0 = black to 100 = white and colour differences (ΔE) between the colour of the red plate ($L=34.64$; $a=56.09$; $b=27.77$) and the colour of the fruit at different stages of maturity which were calculated with the formulae $\Delta E=\sqrt{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2}$ (Hunter, 1975). Total soluble solids were determined by using an Atago N1 (Japan) refractometer at 25°C and the result expressed as Brix. Vitamin C was analysed by titration with potassium iodate and acidity was determined by titration to pH 8.2 with 0.1 N NaOH (Instituto Adolpho Lutz, 1985). Standard procedures (AOAC, 1984) were used for protein determination (Kjeldahl method), pH, ash and moisture. Total sugar values were obtained by titration with Fehling reagents and the results expressed as g of glucose per 100 g for reducing and g of sucrose per 100 g of pulp for non-reducing sugars.

2.4. Gas chromatography

Gas chromatography was carried out using a Hewlett Packard (HP) chromatograph model 5890 fitted with a flame ionization detector (USA) and a LM1 (LM, Brazil) silica capillary apolar column (polymethylsiloxane; 50 m × 0.25 mm, i.d.; 0.5 μm of film thickness). The temperature was held at 40°C for 0.5 min, then programmed to reach 260°C at a rate of 4°C/min. The carrier gas was hydrogen at a flow rate of 40 cm/s. The temperature of the injector and detector were 260 and 280°C, respectively. Retention indices of compounds were calculated on the basis of retention times of normal alkanes (C_6–C_{25}). Injections were made in splitless mode and the sample size for each injection was 1 μl.

2.5. Gas chromatography–mass spectrometry (GC/MS)

A HP mass spectrometer model 5987 (USA) coupled with a HP gas chromatograph model 5987 (USA) fitted with a silica capillary apolar column was used. The chromatographic conditions were the same as before. The ionization voltage was 70 eV and the ion source temperature was 200°C. The sample size used was 3 μl. Identification of compounds in acerola at different stages of maturity was carried out by using GC and MS data from the literature, retention indices and comparison with external standards.

3. Results and discussion

The luminosity of the fruit increased from immature to intermediate mature and showed some degree of fading when reached full maturity as shown by the Hunter values. The visual shift from the green to the red colour is in accordance with the differences observed among the Hunter parameters (Table 1). The colour of the fruits is not only a sign of pigment transformation on the outer surface but it is also related to complex biochemical changes during fruit maturation (Kramer, 1973).

Table 2 shows the variation of some chemical parameters obtained from the acerola fruits at the different stages of maturity. Ascorbic acid was greatly reduced from the green to the red fruit with a loss of about 50%

Table 1
Hunter values of the acerola harvested at different stages of maturity[a,b]

	Immature (green)	Intermediate (yellow)	Mature (red)	MQ
L	27.92b	30.80a	9.34c	1356.5*
a	−8.67c	21.97b	25.97a	3591.2*
b	11.37b	12.13a	−6.34c	1093.0*
ΔE	65.89a	36.20c	48.13b	2009.4*

[a] L, luminosity, 0 = black to 100 = white; a, 80 to 0 = green, 0 to +100 = red; b, 100 to 0 = blue, 0 to +70 = yellow; ΔE, total difference of colour $\sqrt{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2}$, MQ, medium quadratic, "variance analysis", significant at 1% level (*).

[b] According to Tukey test, values in a line followed by different letters are significantly different ($P<0.01$).

Table 2
Characteristics of acerola fruits at different stages of maturity[a]

Characteristics	Stages of maturity		
	Immature (green)	Intermediate (yellow)	Mature (red)
Vitamin C[b]	2164	1065	1074
Protein	1.2	0.9	0.9
Ash	0.4	0.4	0.4
Moisture	91.0	92.4	92.4
Titratable acidity[c]	18.2	15.6	34.4
pH	3.7	3.6	3.7
Soluble solids	7.8	7.7	9.2
Reducing sugars	3.3	4.2	4.4
Non-reducing sugars	1.1	0.1	nd[d]
Total sugars	4.3	4.3	4.4

[a] Results are in g/100 g of sample except as given below.
[b] Results in mg/100 g of sample.
[c] Results in ml of NaOH 0.1 N/100 g of sample.
[d] nd, not detected.

due to biochemical oxidation. This is supported by the appearance of 3-hydroxy-2-pirone which was found only in ripe acerola as result of the oxidative breakdown of ascorbic acid. Soluble solids and total sugars increased from 7.8 to 9.2 °Brix and 3.3 to 4.4% w/w, respectively. The formation of organic acids during maturation was also observed by the correspondent increase in titratable acidity which almost doubled from the immature to the mature stage. Conversely, the pH stayed practically constant, indicating that the differences in degree of dissociation between ascorbic acid which decreased and other organic acids which appeared during maturation, produced a constant net balance. Protein decreased about 30% with maturity due to biochemical degradation and proportional proximate composition distribution. It is well known that amino acids are precursors of a series of volatiles formed during maturation and although this decrease would not have a nutritional impact, it might affect the sensorial characteristics of the fruit due to the formation of volatiles with aroma impact, such as ethyl acetate, (Z)-3-hexenyl acetate, (E)-Z-hexenyl acetate, 2-methyl propyl acetate. These esters were identified in the intermediate and mature but not in the immature fruit studied in the present work, indicating their probable formation from protein breakdown.

Table 3 shows the composition of the volatile fractions. Amongst the components identified in all three stages of maturation are 15 compounds not previously reported in the literature for acerola fruit: 3-hydroxy-2-butanone, 2-methyl-propyl-acetate, (E)-Z-octenal, phenol-2,6 bis (1,1 dimethyl ethyl) 4 methyl, 3,4-didehydro β-ionol, epoxy-β-ionone, heptadecane, tetradecanoic acid, γ-tetradecalactone, hexadecanoic acid, ethyl hexanoate, octadecanoic acid, eicosanoic acid, pentadecanoic acid and 1-octadecanol.

Table 3
Volatile compounds found in immature, intermediate and mature acerola[a,b]

	Compound	*I*	Retention time (min)	Identification		
				Immature (green)	Intermediate (yellow)	Mature (red)
01	Ethyl acetate	600	3.9	b (M)	a,b (M)	b (M)
02	Methyl propyl ketone	674	4.2	a (i)	nd	nd
03	3-Hydroxy-2-butanone	676	4.2	nd	a (i)	a (M)
04	3-Methyl-3 buten-1-ol	717	5.0	nd	a (M)	a (M)
05	Acethyl-methyl-carbinol	700	5.8	nd	nd	b (m)
06	2-Methyl-propyl-acetate	764	6.8	nd	nd	a (m)
07	Isoamyl acetate	865	10.6	nd	b (i)	a,b (M)
08	Methyl hexanoate	906	12.2	nd	a (m)	nd
09	3-Hydroxy-2-pyrone	959	14.5	nd	nd	a (m)
10	3-Octen-1-ol	980	15.3	nd	a,b (i)	nd
11	Ethyl hexanoate	983	15.4	nd	nd	a,b (m)
12	(z)3 Hexenyl acetate	991	15.8	nd	a(m)	a (i)
13	(e)2 Hexenyl acetate	995	16	a (m)	a (i)	a (M)
14	Limonene	1026	17.4	nd	nd	a,b (m)
15	(e)2 Octenal	1030	17.7	nd	nd	a (m)
16	Isoprenyl butyrate	1050	18.3	nd	nd	a (m)
17	Acetofenone	1032	18.5	nd	nd	b (i)
18	Heptanoic acid	–	20.2	nd	nd	b (m)
19	Hexylbutyrate	1177	23.6	nd	a (m)	nd
20	3-Methyl-3-buten hexanoate	1240	26.0	nd	a (m)	a (i)
21	Eugenol	1327	29.3	nd	a,b,c (i)	a,b (M)
22	Vanillin	1354	30.3	nd	a,b (i)	a,b (M)
23	3,4-Dydehydro β-ionol	1396	31.8	a (m)	nd	a (i)
24	Epoxy-β-ionone	1458	34.0	nd	nd	a (m)
25	β-Ionone	1469	34.3	a,b (i)	a,b (i)	a,b (M)
26	Phenol-2,6-bis (1,1 dimethyl ethyl) 4 methyl	–	35.3	c (M)	c (M)	C (M)
27	Dodecanoic acid	1553	37.1	b (I)	b (i)	b (m)
28	4-Hydroxy-β-ionol	1594	38.5	a (m)	a (m)	a (i)
29	Heptadecane	1700	41.6	nd	a,b (m)	a,b (m)
30	Tetradecanoic acid	1755	43.2	b (M)	b (M)	b (i)
31	Pentadecanoic acid	1858	46.1	b (m)	nd	nd
32	γ-Tetradecalactone	1877	46.6	nd	a (m)	a (m)
33	Hexadecanoic acid	1944	48.5	b (M)	a,b,c (M)	a,b,c (M)
34	Ethyl hexanoate	1974	49.2	nd	a (m)	a (i)
35	1-Octadecanol	2040	50.8	c (i)	nd	nd
36	Octadecanoic acid	2137	53.3	b (m)	b,c (m)	b,c (m)
37	Eicosanoic acid	2359	58.5	b,c (m)	b,c (m)	b,c (m)

[a] *I*, retention index, using paraffin (C_6–C_{26}) as references; a, identification by retention index; b, identification by external standard; c, identification by GC/MS; nd, not detected.
[b] Relative quantity: (M), major; (i), intermediate; (m), minor.

Over 65% of the compounds detected in the mature fruit were esters (29%), alcohols (23%) and ketones (16%). These groups of compounds contribute markedly to the fruity note of fruits (Mathesis, Buchanan & Fellman, 1992) and some of the compounds detected in the present work such as ethyl acetate, Z-3-hexenyl

acetate, E-Z-hexenyl acetate, 2-methyl propyl acetate, 3,4-dydehydro β-ionol, epoxy- β-ionone, β-ionone, 4-hydroxy- β-ionol, 3-hydroxy-2-butanone, β3-damascenone are strongly related to the fruit flavour (Winterhalter, Lutz, Herderich & Schreier, 1995; Winterhalter & Schreier, 1995) and, as would be expected, they were all present in the mature acerola.

Some fatty acids were identified in the acerola volatile fraction. This is in agreement with literature data showing that 0.2% w/w of these compounds may be present in the oil fraction of acerola pulp (Miller et al., 1961). Hexadecanoic, octadecanoic and eicosanoic acids were found in the three stages of maturity with the first in relative larger amounts than the others. Tetradecanoic acid was also found in the three stages but gradually decreased during maturation. Conversely, pentadecanoic acid was only detected in the green stage.

The composition of the volatile fractions of the different stages of maturity studied in this work seems to be in accordance with sensory descriptions of other fruits in the literature. During the first stage of maturity, when green odour prevailed, ester concentrations were low. However, their number increased during maturation. They were probably formed from reactions of alcohols and organic acids catalysed by alcohol acyl transferases which are highly active during fruit maturation and appear to have an important role in ester biosynthesis (Olias, Sanz, Rios & Pérez, 1995).

The presence of vanillin and eugenol, detected only in the intermediate and mature stages of the acerola appears to agree with the process of decarboxylation of p-coumaric acid and ferulic acid which occurs during maturation of other fruits to originate these compounds (Buttery, 1971).

The results of the present work show that great modifications occur during acerola maturation and that chemical characterization is important to control the maturation process, both in the plant and during storage.

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