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Total phenolic and carotenoid contents in acerola genotypes harvested at three ripening stages

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Abstract

Acerola fruits (*Malpighia emarginata* D.C.) were harvested from 12 different genotypes cultivated at the Active Germplasm Bank in Federal Rural University of Pernambuco, in the northeast region of Brazil. The effects of maturity stages and weather conditions on total phenolics and carotenoids content were analysed. Mature fruits harvested in the dry season showed the highest and the lowest levels of total phenolics and carotenoids, respectively. During the maturation process, phenolics degradation and carotenoids biosyntheses were observed. Among the acerola genotypes, fruits from genotype number 05 stood out, presenting the highest phenolic and carotenoid contents.

Keywords: Acerola; *Malpighia emarginata* D.C.; Germplasm bank; Fruit ripening; Total phenolics; Total carotenoids

1. Introduction

Researches have shown that fruits and vegetables contain other phytochemicals, in addition to the well-known vitamins C and E or carotenoids, which significantly contribute to their total antioxidant capacity (Gardner, White, McPhail, & Duthie, 2000; Vinson, Hao, Su, & Zubik, 1998). Recent interest in food phenolics has greatly increased on account of the antioxidant and free radical-scavenging abilities associated with some phenolics and their potential effects on human health (Martinez-Valverde, Periago, & Ros, 2000).

Ripening of the fruit involves a series of complex biochemical reactions, such as hydrolysis of starch, production of carotenoids, anthocyanins and phenolics and the formation of volatile compounds (Speirs & Brady, 1991). The nutrient content of freshly harvested edible plants varies, and these variations result from interplay of a number of factors, chiefly genetics, sunlight, reliable rainfall, topography, soils, location, season, fertilisation of soils and maturity (Harris, 1977).

Acerola (*Malpighia emarginata* D.C.), known to be rich in vitamin C, has increasing importance as a crop in the Brazilian northeast, being considered one of the most important natural sources of this vitamin. Due to the need for identifying promising genetic material, the Federal Rural University of Pernambuco (UFRPE) established, in June 1998, an acerola Active Germplasm Bank (AGB). The physical–chemical characteristics of these mature fruits showed vitamin C levels from 1247.10 to 1845.79 mg ascorbic acid 100 ml^{-1} of pulp, total anthocyanins from 3.81 to 47.4 mg 100 g^{-1} of pulp, total flavonols from 7.00 to 18.5 mg quercetin 100 g $pulp^{-1}$, titratable acidity (TA) from 1.04 to 1.87 g of malic acid 100 g^{-1} of pulp, total soluble solids from 7.00 to 8.43°Brix, °Brix/TA ratio from 4.36 to 6.86 and pH from 3.11 to 3.41 (Musser, 2001).

Recently, a number of studies have investigated the phenolic and carotenoid contents in fruits (Prior et al.,

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1998; Rodriguez-Amaya, 1999a). Despite the great commercial potential of the acerola crop, few reports have been published about phytochemical contents and chemical changes during the maturation of this fruit. The purpose of the present study was to investigate the phenolic and carotenoid contents in different acerola genotypes and their changes during the maturation process.

2. Material and methods

2.1. Plant material

Acerola fruits from 12 genotypes of the Active Germplasm Bank of UFRPE were harvested in the dry (November/2001) and rainy (May/2002) seasons. The experimental design of the Bank was randomised in blocks of 12 treatments (genotypes), with five repetitions of one plant in each plot, with a 5×4 m spacing, located at the Sugarcane Experimental Station of Carpina (E.E.C.A.C./UFRPE). The genotypes were codified with the numbers: 02, 03, 04, 05, 06, 07, 08, 11, 12, 13, 14, and 15. The chemical analyses were conducted at the Physical–chemical and Sensorial Food Analysis Laboratory of the Home Sciences Department/UFRPE. Fruits (500 g) were collected of each genotype, and were sorted into three stages of maturation, according to external colour: full green (initial stage), yellow-reddish (half-mature), and red or purple (completely mature, colour characteristic from each genotype). Fruits from each genotype and each maturity stage were crushed in a food processor, and stored at −18 °C prior to analysis.

2.2. Determination of total phenolics

Pulp (1 g) was weighed into beaker, 30 ml of solvent (80% aqueous ethanol, containing 1% conc. HCl) were added, and the sample was extracted using a magnetic mixer for 20 min in the absence of light at room temperature (25 °C) and filtered. The extraction process was repeated three times. The resultant ethanolic extracts were combined to a final volume of 100 ml. The total phenolics content was measured spectrophotometrically at 725 nm using the Folin–Ciocalteu reagent (Wettasinghe & Shahidi, 1999). Results were expressed as catechin equivalents (mg 100 g^{-1}) of fresh weight.

2.3. Determination of total carotenoids

A weight portion (1–10 g) was used to measure the total carotenoids. The pulp obtained from fruits at full green and yellow-reddish colours were saponified to remove the chlorophylls. Subsequently, the carotenoids were extracted according to Rodriguez-Amaya (1999b). The total carotenoids content was measured spectrophotometrically at 450 nm using the extinction coefficient of 2500 and results were expressed as β-carotene equivalents (μg g^{-1}) of fresh weight (Gross, 1987).

2.4. Statistical analysis

At least three replications of the experiment were performed. Total phenolics and carotenoids data were analysed by the Tukey test, in order to compare the different genotypes, and by *t*-Student test, to verify whether there were significant differences in each season, at the same stage of maturation.

3. Results and discussion

The phenolic compound concentration in acerola fruit at three maturity stages, harvested in dry and rainy seasons are shown in Table 1. This phytochemical content decreased during maturation, although there were non-linear changes to 02, 03, 14 and 15 genotypes. Similar results were reported by Gao, Ohlander, Jeppsson, Björk, and Trajkovski (2000), who attributed non-linear changes to three cultivars of sea buckthorn on climate or some physiological factors. The phenolic level also decreased during the maturation process in pear fruit (Amiot, Tacchini, Aubert, & Oleszek, 1995) and in apple fruit (Burda, Oleszek, & Lee, 1990).

The highest phenolics content was found in mature fruits of the genotypes 07, 12 and 13, which were harvested in the dry season. These values did not differ significantly from genotypes 05, 06, and 14. According to Musser (2001), the fruits from genotype 05 showed the highest total anthocyanin and flavonol contents, both belonging to the phenolic group. Thus, there are other phenolic compounds present in the analysed genotypes that may justify this result.

Mature fruits harvested in the dry season showed higher phenolic contents than those harvested in the rainy season. However, this increase was significant to genotypes 06, 07, 08, 12, 13, and 14. Esteban, Villanueva, and Lissarrague (2001) reported that total phenolics content decreased for the irrigated grapes, especially at the final ripening. This result was, probably, influenced by climatic factors since the rainfall may dilute the cellular juice and reduce the total phenolic levels.

Table 2 shows the total carotenoid content in acerola fruit at three maturity stages, harvested in the dry and rainy seasons. The levels of this phytochemical were very low in green fruit and then greatly increased in the mature fruit. These changes reflect degradation of chlorophylls with a concomitant rise in carotenoids (Alves, Chitarra, & Chitarra, 1995). In some mature fruits, such as strawberry (Woodward, 1972), red currant (Gross, 1982/1983), and olive Picual variety (Roca

Table 1
Total phenolics content in acerola fruits at three maturation stages harvested in dry and rainy seasons[a]

Genotypes	Stages of maturity					
	Green		Half-mature		Mature	
	Dry season	Rainy season	Dry season	Rainy season	Dry season	Rainy season
02	$3109^{bd\ A}$	$2372^{cd\ A}$	$1288^{e\ A}$	$722^{d\ A}$	$1272^{bc\ A}$	$1214^{bc\ A}$
03	$2002^{cd\ B}$	$2418^{cd\ A}$	$2595^{bc\ A}$	$1321^{bcd\ B}$	$1024^{cd\ A}$	$952^{de\ A}$
04	4098^{aA}	$2978^{bc\ B}$	$3378^{a\ A}$	$2206^{a\ B}$	$1354^{cb\ A}$	$1248^{bc\ A}$
05	$3517^{ab\ A}$	$3822^{a\ A}$	$2920^{b\ A}$	$1884^{ab\ B}$	$1508^{ab\ A}$	$1653^{a\ A}$
06	$2997^{cb\ A}$	$3343^{ab\ A}$	$1933^{d\ A}$	$1643^{abc\ B}$	$1703^{ab\ A}$	$930^{de\ B}$
07	$3815^{ab\ A}$	$2934^{bc\ A}$	$2449^{c\ A}$	$2049^{a\ B}$	$1809^{a\ A}$	$1502^{a\ B}$
08	$3626^{ab\ A}$	$3291^{ab\ A}$	$2298^{cd\ A}$	$1927^{ab\ A}$	$1399^{bc\ A}$	$1032^{cd\ B}$
11	$4363^{ab\ A}$	$3274^{ab\ B}$	$2445^{c\ A}$	$2081^{a\ A}$	$1196^{cd\ A}$	$1183^{bc\ A}$
12	$3487^{b\ A}$	$3954^{a\ A}$	$2684^{bc\ A}$	$1955^{ab\ B}$	$1888^{a\ A}$	$737^{e\ B}$
13	$4524^{a\ A}$	$3043^{bc\ B}$	$3703^{a\ A}$	$2163^{a\ B}$	$1888^{a\ A}$	$1220^{bc\ B}$
14	$2620^{c\ A}$	$2512^{cd\ A}$	$706^{f\ B}$	$2162^{a\ A}$	$1720^{ab\ A}$	$1361^{b\ B}$
15	$2152^{dc\ A}$	$1945^{d\ A}$	$536^{f\ B}$	$1153^{cd\ A}$	$896^{d\ A}$	$841^{de\ A}$

[a] mg 100 g^{-1} of catechin equivalents (fresh weight).
[b] Means values with the same lowercase letters in the same column are not significantly different by Tukey test ($P > 0.05$).
[c] Means values with the same capital letters in a row, considering dry and rainy season with the same stage maturation, are not significantly different by *t*-Student test ($P > 0.05$).

Table 2
Total carotenoids content in acerola fruits at three maturation stages harvested in dry and rainy seasons[a]

Genotypes	Stages of maturity					
	Green		Half-mature		Mature	
	Dry season	Rainy season	Dry season	Rainy season	Dry season	Rainy season
02	$0.72^{cd\ A}$	$1.00^{d\ A}$	3.13^{bA}	$3.25^{b\ A}$	$15.0^{e\ B}$	$17.2^{f\ A}$
03	$1.11^{cd\ A}$	$1.67^{bcd\ A}$	$1.64^{d\ A}$	$1.47^{d\ A}$	$9.4^{f\ B}$	$14.1^{g\ A}$
04	$3.23^{a\ A}$	$1.65^{bcd\ B}$	$3.09^{b\ A}$	$3.39^{b\ A}$	$14.4^{e\ B}$	$23.5^{d\ A}$
05	$2.16^{b\ B}$	$3.52^{a\ A}$	$4.19^{a\ B}$	$5.89^{a\ A}$	$30.9^{a\ A}$	$20.4^{e\ B}$
06	$2.27^{b\ A}$	$2.00^{bc\ A}$	$1.87^{cd\ A}$	$1.95^{d\ A}$	$17.9^{d\ B}$	$30.0^{b\ A}$
07	$1.47^{bc\ B}$	$2.48^{b\ A}$	$2.59^{b\ B}$	$3.01^{b\ A}$	$12.5^{f\ B}$	$24.7^{cd\ A}$
08	$0.43^{d\ B}$	$1.36^{cd\ A}$	$0.75^{e\ B}$	$1.47^{d\ A}$	$20.7^{c\ B}$	$27.4^{b\ A}$
11	$1.36^{c\ A}$	$1.36^{cd\ A}$	$1.41^{d\ A}$	$1.40^{d\ A}$	$21.8^{c\ B}$	$27.0^{c\ A}$
12	$1.13^{cd\ B}$	$2.31^{bc\ A}$	$1.81^{cd\ B}$	$2.40^{bc\ A}$	$19.4^{cd\ B}$	$16.4^{f\ A}$
13	$0.91^{cd\ B}$	$2.00^{bc\ A}$	$2.32^{c\ A}$	$2.03^{cd\ A}$	$26.6^{b\ B}$	$40.6^{a\ A}$
14	$0.32^{d\ B}$	$2.29^{bc\ A}$	$1.95^{cd\ B}$	$2.40^{bc\ A}$	$11.8^{f\ B}$	$16.9^{f\ A}$
15	$1.57^{bc\ B}$	$2.37^{b\ A}$	$1.81^{cd\ B}$	$2.85^{b\ A}$	$20.9^{c\ A}$	$22.5^{de\ A}$

Means values with the same lowercase letters in the same column are not significantly different by Tukey test ($P > 0.05$).
Means values with the same capital letters in a row, considering dry and rainy season with the same stage maturation, are not significantly different by *t*-Student test ($P > 0.05$).
[a] μg g^{-1} of β-carotene equivalents (fresh weight).

& Mínguez-Mosquera, 2001), in which the red colour is due to the presence of anthocyanins, carotenoid contents decrease during ripening. Also acerola red colour is due to anthocyanin pigments; however, this decrease did not occur. A similar result was reported by Roca and Mínguez-Mosquera (2001) for olive Aberquina variety, which showed increase of carotenoid pigments during the ripeness state.

Among mature fruits harvested in the dry season, those from genotype 05 showed the highest total carotenoid content. Rodriguez-Amaya (1999a) has reported carotenoid levels for acerola from Pernambuco, Ceará and São Paulo states, in Brazil, of 30.1, 24.1 and 4.5 μg g^{-1}, respectively. These data show that the chemical composition can vary according to environmental conditions, harvest season, varieties, and also on stage of maturity.

Carotenoid content was higher in mature fruits harvested in the rainy season than in those harvested in the dry season, except for the genotypes 05 and 12. Among the fruits harvested in the rainy season, those from genotype 13 showed the highest carotenoid concentration. Three months before these fruits were harvested, the field was fertilised. According to Gross (1987), soil fertilisation is one of the factors that affect carotenoids biosyntheses in fruit. This fact, probably, contributed to the highest level of carotenoids, for fruits harvested in the rainy season.

The variations in total carotenoid and phenolic levels observed among the genotypes studied can be attributed

to genetic factors. According to Macheix, Fleuriet, and Billot (1990), there are generally fairly good correlations between the phenolic levels during ripening and the activity of the enzymes responsible for biosynthesis and degradation of these constituents. Some of these enzymes, such as phenylalanine ammonialyase (PAL) and hydroxycinnamate CoA ligase (CoAL), emerge as key parameters in the quantitative expression or the orientation of the metabolism. The regulation of the carotenoid biosynthesis is complex and restricted to specific tissues where they are used. Enzymes of carotenoid biosynthesis, such as geranylgeranyl pyrophosphate (GGPP) synthase, are functional in the chloroplast/chromoplast but are codified by nuclear genes (Delgado-Vargas, Jiménez, & Paredes-López, 2000).

In conclusion, acerola fruits from twelve genotypes showed different total phenolic and carotenoid contents. During the maturation process, phenolics degradation and carotenoids biosynthesis were observed. Among the acerola genotypes, fruits from 05 genotype stood out, presenting the highest phenolic and carotenoid contents.

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