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Ascorbic acid in exotic fruits: a liquid chromatographic investigation

Giuliana Vinci, Francesco Botrè, Giustino Mele

Instituto di Merceologia, Università 'La Sapienza', Via del Castro Laurenziano 9, 00161 Rome, Italy

&

Giovanni Ruggieri*

Cattedra di Merceologia, Dipartimento di Economia, III Università di Roma, Via Ostiense 139, 00154 Rome, Italy

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The levels of ascorbic acid (AA) have been measured by means of an HPLC method in 11 different exotic fruits (avocado pear, babaco, feijoa, grapefruit, kiwi, kumquat, litchi, mango, papaya, passion fruit, pineapple) and, for comparative purposes, in two citrus fruits (lemon and orange). They were measured in the exotic fruits at two different stages of ripening: (i) immediately after purchasing from a local fresh fruit market, and (ii) after a one-week period of artificial ripening. The results show that all tropical fruits contain relatively high levels of AA (varying between 20 and 90 mg/100g), with the exceptions of avocado pear and feijoa (whose AA levels are markedly affected by oxidation processes). Moreover, the results show that there is a remarkable loss of AA content (usually 30–40%) after the one-week period of artificial ripening, in all the different tropical fruits considered. They seem to indicate that the process of artificial ripening, which is usually carried out during the short-term storage of exotic fruits, can affect the nutritional value of this kind of food as far as the concentration of the reduced form of vitamin C is concerned.

INTRODUCTION

Exotic fruits. whose use was once restricted to people living in limited geographic areas, have become quite a common food in non-exotic countries. These fruits are gaining a prominent role in the diet due to their nutritional value and to the generally high content of hydrosoluble vitamins, especially vitamin C. It follows that the determination of ascorbic acid in all these fruits would be of great interest in the field of food chemistry (Liao & Seib 1987; Conticini, 1991).

The cultivation of tropical fruits, favored by suitable climatic conditions, has become more and more diffused in Italy, especially in the southern regions, whose geographic and climatic conditions are very suitable for their successful cultivation.

Many analytical methods, based on different techniques, are reported in the literature for the quantitative determination of vitamin C. The usual methods include titration (AOAC, 1980), colorimetry (Roe & Kuethe, 1943; Bajaj & Kaur, 1981), electrochemistry (Oi-Wah Lau *et al.*, 1989); chromatography (Pachla & Kissinger, 1976; Sood *et al.*, 1976; Finley & Duano, 1981; Speed *et al.*, 1984; Kissinger & Pachla, 1987; Graham & Annette, 1992; Kishida *et al.*, 1992; Nisperos-Carriedo *et al.*, 1992) and enzymatic assays (Uchiyama *et al.*, 1991; Lorenti *et al.*, 1992).

Some of the above-mentioned methods present drawbacks such as, for example, the titrimetic one (AOAC, 1980) by the use of 2,6-dichloroindophenol (DCIP), which is not applicable in the presence of reducing agents (i.e. Fe^{2+}, Cu^{2+}, SO_2, sulfite, and thiosulphate ions) or in colored solutions. The colorimetric methods (Roe & Kuethe, 1943; Bajaj & Kaur, 1981) show some disadvantages because they are endowed with a relatively low sensitivity and require a long procedure. Electrochemical methods have been available for many years (Oi-Wah Lau *et al.*, 1989), but they lack sufficient selectivity to be used for trace levels of the vitamin in complex biological media. High performance liquid

*To whom correspondence should be addressed.

chromatography (HPLC) techniques have also been introduced to determine ascorbic acid, as these methods allow increases in both the specificity and the sensitivity of the assay.

In the present study the application of HPLC to the determination of AA levels in some exotic fruits is described. The method proposed here is similar to one reported in the literature (Sood *et al.*, 1976), but some variations, regarding mainly the chromatographic conditions and the sample pretreatment operations, have been introduced in order to optimize and to make easier the overall procedure.

MATERIALS AND METHODS

Instrumentation

The HPLC system was constituted by a model 5000 Liquid Chromatography pump (Varian Associates, Inc. Palo Alto, CA, USA) connected to a 235C diode array detector (Perkin–Elmer, Norwalk, CT, USA). The column was a Supelcosil LC-18 (25 cm × 4·6 mm i.d., 5 μm particle size) (Supelco Inc., Bellefont, PA, USA). The data were stored and processed by a PE Nelson Model 1020 Personal Integrator (PE Nelson, Norwalk, CT, USA); the chromatograms were recorded by an Epson LX 400 dot-matrix printer (Epson Italia SPA, Milano, Italy). Homogenization of the edible part of the fruit samples was carried out by means of a laboratory-scale homogenizator (Universal Laboratory AID, type MPW-309).

Materials

Avocado pear, babaco, feijoa, grapefruit, kiwi, kumquat, litchi, mango, papaya, passion fruit and pineapple fruits were purchased from local fruit markets and stored at 4°C (humidity 50–55%). Samples were not completely ripe at the time of purchase. One half of the fruit sample purchased was stored at room temperature in a sealed nylon bag for one week to allow artificial ripening.

Ascorbic acid (99·7% purity), tetrabutylammonium hydroxide (solution 20% in water), formic acid (98% purity) and acetonitrile gradient grade for chromatography (Lichrosolv) were purchased from Merck (Darmstadt, Germany) and water ultrapure grade by Milli-Q from Millipore (Millipore Corporation, USA).

Preparation of fruit samples

All fruits were peeled, and a portion (100 g) of the edible part was cut in small pieces and homogenized by a rotating blade (≈ 1000 rpm for 20–30 s), at room temperature, in a dark vessel; a portion of the homogenate (15–25 g, accurately weighed) of this slurry was transferred to a 100-ml dark volumetric flask and diluted to volume with double distilled water. The flask was sonicated for 5 min, the resulting mixture was then centrifuged at 5000 rpm for 10 min (T = 20°C). The volume of the supernatant after centrifugation was always accurately measured. A fraction of the supernatant was filtered through a 0·4-μm membrane filter. Portions of the extract (10 μl) were injected into the HPLC chromatograph.

Operative conditions in the chromatographic analyses

A binary solvent system of tetrabutyl ammonium hydroxide (2×10^{-3} M, pH = 5·0 by 1% formic acid)/acetonitrile (75:25, v/v) was used at a flow rate of 0·8 ml/min. Detection of AA was carried out at λ = 254 nm. AA was identified by comparing its retention time with that of a reference standard.

A stock AA standard solution (1 mg/ml) was prepared daily in double distilled water and diluted to give 0·1, 0·01, 0·005 and 0·001 mg/ml working standard solutions. The stock and working AA solutions were stable for one day if stored in the dark at $T = 4 \pm 1$°C.

The analytical recovery was evaluated in all cases by adding a known amount of AA standard (at known concentration) to each sample of fruit and analyzing the resulting solution. The internal standard of AA was added (i) just before the homogenization step, (ii) just before the sonication step, (iii) just before the centrifugation step, and (iv) after the centrifugation step. No differences among these four procedures were detected. All results listed in Table 2 refer to tests carried out by adding the AA standard solution prior to the centrifugation step.

RESULTS AND DISCUSSION

Table 1 shows the levels of AA measured by the HPLC method here described in the 11 exotic fruits and, for comparison purposes, in two reference fruits (orange and lemon). It also shows the loss of AA after the one-week period of artificial ripening detected in the 11 exotic fruits. Table 2 shows the analytical recovery of AA in the 11 exotic fruits, measured after the artificial

Table 1. Content of ascorbic acid in 11 different exotic fruits at two stages of ripeness

Fruit	[AA] (fresh) (mg/100 g)[a]	[AA] (artificially ripened) (mg/100 g)[a]	Loss (%)
Avocado pear	10·23[b]	2·80[b]	72·8[b]
Babaco	31·20	18·06	42·1
Feijoa	n.d.[b]	n.d.[b]	n.d.[b]
Grapefruit	64·78	45·08	30·4
Kiwi	67·23	45·93	31·7
Kumquat	55·29	37·23	32·7
Litchi	21·94	13·08	40·4
Mango	25·32	16·38	40·4
Papaya	88·20	53·78	39·0
Passion fruit	64·78	39·36	39·2
Pineapple	30·60	18·12	40·8
Lemon	51·30	—	—
Orange	49·80	—	—

[a]Considering only the edible part of the fruit.
[b]Not detectable with precision because of oxidation processes.

Table 2. Analytical recovery of ascorbic acid in 11 different exotic fruits

Fruit	[AA] found (mg/100 g)[a]	[AA] added (mg/100 g)[a]	Total found value (mg/100 g)[a]	SD	CV (%)	Recovery (%)
Avocado pear	2·80[b]	15·20	15·00[b]	1·39	2·1	83·3[b]
Babaco	18·06	35·20	51·50	1·53	3·3	96·7
Feijoa	n·d·[b]	40·00	n·d·[b]	—	—	n·d·[b]
Grapefruit	45·08	90·40	136·53	2·59	1·9	100·8
Kiwi	45·93	87·40	131·35	2·36	1·8	98·5
Kumquat	37·23	92·50	124·83	2·75	2·2	96·2
Litchi	13·08	33·30	46·80	1·09	2·5	100·9
Mango	16·38	40·70	55·60	1·89	3·4	97·4
Papaya	53·78	91·30	147·00	2·05	1·4	101·3
Passion fruit	39·36	88·20	124·10	3·07	2·5	97·3
Pineapple	18·12	50·40	66·40	1·39	2·1	96·9

[a]Considering only the edible part of the fruit.
[b]Not detectable with precision because of oxidation processes.

ripening treatment. The AA concentration values listed in Tables 1 and 2 represent the average data obtained from four different fruits (three determinations each fruit).

Figure 1 shows a representative chromatogram (respective to the babaco fruit). It shows that the chromatographic operative conditions fixed for the present study allowed a good resolution of the AA peak in a short time (retention time = 6·7 min): no peaks due to interferences were observed in the chromatograms of all 13 fruit samples. The chromatogram represented in Fig. 1 also shows the decrease of AA after the period of artificial ripening. The decrease in AA levels, consequent to the artificial ripening treatment, varies in average between 32 and 42% with a maximum of 73% for the avocado pear.

From Tables 1 and 2, it can be observed that only two fruits (feijoa and avocado pear) show an anomalous behavior. It must be noticed that the AA content is not detectable in the feijoa: the AA levels in the fruits prior to the artificial ripening treatment are lower than the sensitivity limits of the method (1 ng/μl, considering the solution obtained at the end of the sample pretreatment, after filtration). Moreover, to assess the AA levels in the feijoa, the addition method has also been used; the obtained recoveries listed in Table 2 show that, again, no AA was found, not even the exogenous one. The complete absence of AA could be ascribed either to oxidation processes induced by free metal ions and/or to specific enzymes present inside the feijoa fruit that take part in the metabolism of ascorbic acid (probably the enzyme ascorbate oxidase) (Lorenti *et al.*, 1992).

From the results listed in Table 1, it can be observed that the ascorbic acid loss in the avocado pear fruit is more than the average loss in the other fruits. For this fruit the ascorbic acid content decreases very rapidly in time in the same sample, so that the values reported in the tables refer only to the first assay. In Table 2 a very low recovery is reported, which is also probably due to the presence of free metal ions and/or of oxidative enzymes oxidizing the ascorbic acid, as evidenced by the addition method. To three portions of the same avocado pear sample, three different ascorbic acid quantities were added. Each portion was then analyzed by HPLC at different time intervals (Fig. 2).

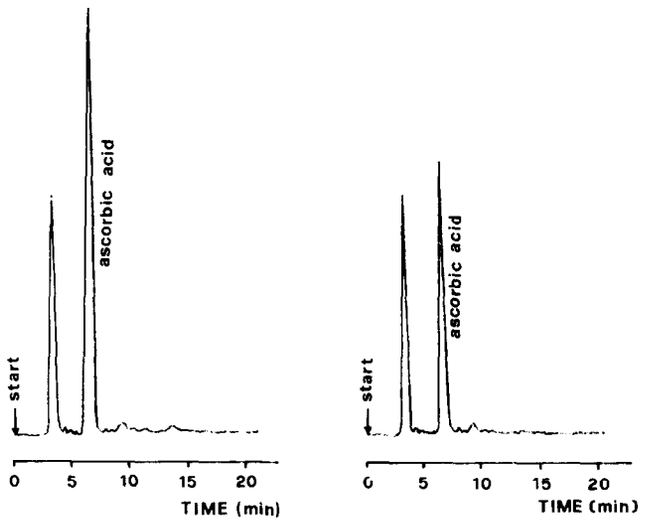

Fig. 1. Chromatograms of a fruit sample (babaco): (a) prior and (b) after the artificial ripening treatment.

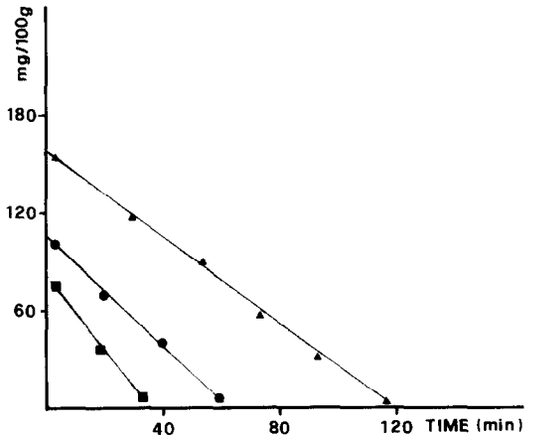

Fig. 2. Variations of ascorbic acid content, as a function of time, in some samples of avocado pear fruit at three levels of ascorbic acid added ▲ = 150 mg/100 g; ● = 100 mg/100 g; ■ = 70 mg/100 g).

CONCLUSIONS

The investigation carried out on several types of exotic fruits shows that a relevant amount of vitamin C is present in some of them (babaco, grape fruit, kiwi, kumquat, papaya, passion fruit, pineapple); therefore, these particular fruits could be considered as an additional source of vitamin C in the diet or as a substitute for the traditional citrus fruit.

Furthermore, it seems worthwhile to highlight the marked decrease in the levels of ascorbic acid which has been detected, in all samples, following the treatment of artificial ripeness. This evidence shows that the nutritional value of a naturally ripe fruit is not the same than of an artificially ripe one. This finding also stresses the primary role played by the storage conditions on the nutritional values of exotic fruits.

Additional experiments are presently in progress, in order to further evaluate the entity of AA losses as a function of the storage conditions and to clarify the nature of the AA oxidation processes evidenced in the present study.

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