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Characterisation of the Carotenoids and Assessment of the Vitamin A Value of Brasilian Guavas (*Psidium guajava* L.)

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ABSTRACT

The carotenoids of guava cultivar IAC-4 from the State of São Paulo (southeastern Brasil) were identified as β-carotene, ζ-carotene, γ-carotene, zeinoxanthin, lycopene, 5,6,5′,6′-diepoxy-β-carotene and 5,8-epoxy-3,3′,4-trihydroxy β-carotene. The principal pigment was lycopene, corresponding to 86% of the total carotenoid content (62 μg/g). β-carotene was present at 3·7 μg/g; consequently, the vitamin A was relatively low (617 IU/100g). The same carotenoids were encountered in guavas from the States of Ceará and Pernambuco (northeastern Brasil). Cis-*γ-carotene and 5,8-epoxy-zeinoxanthin were also found in the samples from Pernambuco. While the lycopene contents of the northeastern fruits were equal to, or lower than, that found in guava IAC-4, the β-carotene level (5·5–11·9 μg/g) was higher, corresponding to higher vitamin A values (917–1983 IU/100 g). With respect to vitamin C, the amount detected in guava cultivar IAC-4 was much higher (97·7 mg/100 g) than those encountered in the northeastern guavas (9·2–52·2 mg/100 g).*

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INTRODUCTION

Although guava (*Psidium guajava* L.) is one of the better known and more widely available tropical fruits, its carotenoid composition has not been established. Nakasone *et al.* (1976) quantified only the lycopene fraction while Nogueira *et al.* (1978) and Fonseca *et al.* (1969) determined the β-carotene content. Without giving the reference, Holdsworth (1979) cited α-carotene and β-carotene as the principal pigments of guava.

The present paper deals with the characterisation of the carotenoids and assessment of the vitamin A value of Brasilian guavas as part of our overall research program to study Brasilian fruits. Our studies involve the separation, identification and quantitation of individual carotenoids. The vitamin A value is then calculated taking into account only the active carotenoids with their corresponding vitamin A activities. This procedure avoids the error in existing data, based on the total carotenoid content, which could include inactive carotenoids and thus lead to grossly inaccurate values. The need for the re-evaluation of the vitamin A values of foods has been emphasized by workers in the field (Klaui & Bauernfeind, 1981). This is especially important in Brasil and other developing countries where vitamin A deficiency is considered a serious nutritional problem. Renewed interest on carotenoids and vitamin A has also been demonstrated in recent years because of their possible inhibitory effect on certain types of cancer (NRC, 1982).

MATERIALS AND METHODS

Materials

Ripe guavas of the cultivar IAC-4 were collected at random from a commercial plantation in the State of São Paulo and transported immediately to our laboratory. One collection was made in 1981 and another in 1982. The fruits had uniform colour, size (diameter, 5·3 cm) and weight (88 g).

The cultivar IAC-4, developed by the Instituto Agronômico de Campinas, accounts for 90% of commercial guava production in the State of São Paulo (southeastern Brasil) and is increasingly grown commercially in other states. It produces round fruits with yellow skin and vivid pink pulp, utilized industrially for making canned guava slices

and *goiabada*, a well-liked traditional dessert. Our previous paper (Padula & Rodriguez-Amaya, 1983) showed that these fruits serve as excellent raw materials for commercial juice production.

Ripe guavas from northeastern Brasil (Pernambuco and Ceará) were bought at different times (1981–1982) at farmers' markets and transported by air to Campinas. Three lots of round-shaped guavas from Pernambuco were analysed. Of the guavas from Ceará, one lot was round-shaped and two, pear shaped. All guavas had deep pink inner flesh. The pear-shaped guavas had a greener tinge than the yellow-skinned round shaped fruits. The fruits differed in size and weight and were smaller than the IAC-4 guavas. At the present time, guava juice is commercially manufactured in northeastern Brasil, from a mixture of unselected guava varieties, for distribution throughout the country.

For the quantitative composition of the carotenoids, lots consisting of six fruits were analysed. The fruits from each lot were quartered and two opposite sections from each fruit were combined and homogenized in a Waring blender. One hundred gram portions were taken for analysis.

Carotenoid and other determinations

The carotenoid composition was determined according to procedures described previously (Rodriguez *et al.*, 1976). Since lycopene was present in amounts disproportionately much higher and sufficient amount of sample had to be taken to permit detection of the other pigments, lycopene overloaded the column. Thus, all fractions obtained from the initial MgO:HyfloSupercel column had to be rechromatographed on an alumina column. Vitamin A values were calculated according to NAS-NRC (1980), using the provitamin A activities tabulated by Bauernfeind (1972).

All other determinations were undertaken using standard procedures of the AOAC (1980). Vitamin C was determined by the microfluorometric method, AOAC 43.061.

RESULTS AND DISCUSSION

Carotenoid composition of guava cultivar IAC-4

Seven carotenoids were detected in the guava cultivar IAC-4. The carotenes were identified by the visible absorption spectra and

chromatographic behaviour as β-carotene, ζ-carotene, γ-carotene and lycopene.

A pigment, which eluted before lycopene from the MgO:Hyflo-Supercel column, exhibited an absorption spectrum similar to that of α-carotene. Its R_F value of 0·60 on the silica gel plates developed with 3% methanol in benzene indicated the presence of a hydroxyl group. This was confirmed by the positive reaction to acetylation with acetic anhydride. Reaction to methylation with acidified methanol, however, was negative, demonstrating that the hydroxyl substituent was not located in an allylic position. It was thus identified as zeinoxanthin.

A carotenoid which eluted jointly with γ-carotene from the MgO: HyfloSupercel column and was subsequently separated on an alumina column, was identified as 5,6,5′,6′-diepoxy-β-carotene. Its epoxy nature was first shown by the transformation of its yellow colour to blue on exposure of the silica gel plates to HCl vapour. The visible absorption spectrum in light petroleum was similar to β-carotene in shape but with the maxima 7 nm lower. Addition of a few drops of 0·1N HCl to the pigment dissolved in ethanol produced a hypsochromic shift of 40 nm, confirming the presence of two 5,6-epoxy substituents.

The most polar pigment was identified as 5,8-epoxy-3,3′,4-trihydroxy-β-carotene. From its behaviour on the column and its position on the TLC plates (origin), it appeared to be trihydroxy carotenoid. It responded positively to acetylation with acetic anhydride, confirming the presence of the hydroxyl functions. Response to methylation with acidified methanol was also positive, demonstrating the allylic position of one of these substituents. The presence of an epoxy group was first revealed by exposure of the TLC plates to HCl gas which changed the yellow colour to blue–green. The visible spectrum (λ_{max}, (410), 432, 459 nm in light petroleum) was consistent with a β-carotene derivative containing a 5,8-epoxy group. Thus, all the properties agreed with the identification of this pigment as 5,8-epoxy-3,3′,4-trihydroxy-β-carotene. Since it is a carotenoid hitherto unreported in the literature, mass spectrometric confirmation would be necessary. In any case, it does not make a significant contribution to the colour and has no vitamin A activity.

Lycopene constituted 86% of the total carotenoid content (Table 1). Thus, in spite of the large amount of carotenoids present, the vitamin A value was low, being due solely to the small amounts of β-carotene.

The β-carotene content obtained in this study is slightly higher than the

TABLE 1
Carotenoid Content (μg/g) and Vitamin A Value (IU/100 g) of the Guava Cultivar IAC-4[a]

Carotenoid/Vitamin value	*Whole fruit*	*Peeled fruit*
β-carotene	3·7 ± 0·7	5·0 ± 1·2
ζ-carotene	trace	trace
γ-carotene	trace	—
Zeinoxanthin	1·0 ± 0·6	0·5 ± 0·1
Lycopene[b]	53·4 ± 6·3	56·6 ± 6·4
5,6,5′,6′-diepoxy-β-carotene	trace	0·1 ± 0·2
5,8-epoxy-3,3′,4-trihydroxy-β-carotene[c]	4·0 ± 0·3	2·0 ± 0·2
Total	62·1 ± 7·8	64·2 ± 4·9
Vitamin A value	617 ± 112	833 ± 200

[a] Results based on the separate analyses of four lots of whole fruits and two lots of peeled fruits. Each lot consisted of six fruits.
[b] The lycopene fraction contained small amounts of *cis*-lycopene.
[c] Tentative identification. Approximate value calculated on the basis of the β-carotene absorptivity.

3·0 μg/g encountered by Nogueira *et al.* (1978) also for guava IAC-4, but obviously much lower than that reported by Fonseca *et al.* (1969) for red guava, also from São Paulo.

Since many Brasilians peel the guava when eaten as is, it was of interest to find out if such a practice reduced the β-carotene, already present in low levels. Table 1 shows that this was not the case, at least with guava cultivar IAC-4. The β-carotene concentration was, in fact, higher, thus giving a higher vitamin A value for the peeled fruit. This contradicts the data given in the INCAP-ICNND food composition Table for Latin American countries (INCAP-ICNND, 1961) where the vitamin A value for the pulp (70 μg/100 g) was lower than for the whole fruit (80 μg/100 g).

Comparison with guavas from northeastern Brasil

Since commercial guava juice in Brasil is manufactured in the northeast, some samples from this region were also analysed. Because of the distance and the difficulty in obtaining the samples, only a limited number was analysed. In any case, for as long as the cultivars are not defined, no meaningful data on their composition could be obtained. The heterogeneity of samples could be deduced from the great variation in the carotenoid composition (Table 2). In all cases, however, the samples from

TABLE 2
Carotenoid Content (μg/g) and Vitamin A Value (IU/100 g) of Guavas from Northeastern Brasil[a]

Carotenoid/Vitamin value	*Guavas from Pernambuco*	*Guavas from Ceará*	
		Round shaped	*Pear shaped*
β-carotene	11·9 ± 5·2	6·6	5·5 ± 2·3
ζ-carotene	Trace	Trace	Trace
Cis-γ-carotene	Trace	—	—
γ-carotene	0·4 ± 0·3	0·4	Trace
Zeinoxanthin	1·9 ± 0·7	1·9	1·5 ± 0·2
Lycopene[b]	53·4 ± 14·1	48·5	47·0 ± 15·7
5,6,5′,6′-diepoxy-β-carotene	0·1 ± 0·1	0·6	0·3 ± 0·4
5,8-epoxy-zeinoxanthin	0·2 ± 0·1	—	—
5,8-epoxy-3,3′,4-trihydroxy-β-carotene[c]	2·1 ± 1·9	4·0	2·3 ± 0·7
Total	70·0 ± 19·5	62·0	56·6 ± 16·5
Vitamin A value	1983 ± 872	1100	917 ± 377

[a] Results based on the separate analyses of three lots from Pernambuco and three lots from Ceará (one round shaped and two pear shaped). Each lot consisted of six fruits.
[b] The lycopene fraction contained small amounts of *cis*-lycopene.
[c] Tentative identification. Approximate value calculated on the basis of the β-carotene absorptivity.

northeastern Brasil, where the climate is much warmer, were higher in β-carotene. The lycopene content of fruits from Pernambuco was comparable with, while that of the Ceará fruits was lower than, the amount found in IAC-4 guavas.

The vitamin A values were consequently higher for the northeastern fruits (917–1983 IU/100 g) than for IAC-4 fruits (617 IU/100 g). These are equivalent to 330–595 and 185 μg/100 g, respectively, and thus higher than the 109 μg/100 g reported for Hawaiian guavas (Wenkam & Miller, 1965) and the 80 μg/100 g quoted by INCAP-ICNND (1961) but much lower than the 4170 IU reported by Moura Campos (1958) for Brasilian guava.

Two other pigments were detected in guavas from Pernambuco: *cis*-γ-carotene and 5,8-zeinoxanthin. The former eluted before γ-carotene and exhibited absorption maxima 4 nm lower than γ-carotene (*trans*). On iodine-catalysed isomerisation, a bathochromic shift was observed, consistent with the conversion of a *cis* carotenoid to the *trans* isomer. The

TABLE 3
Comparison of Some General Properties of Guava Cultivar IAC-4 with Guavas from Northeastern Brasil

	Cultivar IAC-4	*Guavas from Pernambuco*	*Guavas from Ceará*	
			Round shaped	*Pear shaped*
°Brix (20°C)	9·8 ± 0·5	10·1 ± 0·1	10·6	7·0 ± 1·1
pH	4·1 ± 0·1	3·7 ± 0·0	3·2	3·1 ± 0·5
Titrable acidity (% citric acid)	0·4 ± 0·1	0·7 ± 0·0	0·4	0·3 ± 0·0
Reducing sugar (%)	6·7 ± 0·8[b]	5·0 ± 0·1	8·2	4·9 ± 1·2
Total sugar (%)	7·0 ± 1·1[b]	5·3 ± 0·1	8·1	4·9 ± 1·3
Vitamin C (mg/100 g)	97·7 ± 12·0	52·2 ± 15·3	38·2	9·2 ± 1·7
Moisture	83·3 ± 1·6	77·8 ± 2·3	82·0	86·4 ± 0·8

[a] Results based on the separate analyses of four lots of guava cultivar IAC-4, three lots from Pernambuco and three lots from Ceará (one round shaped and two pear shaped). Each lot consisted of six fruits.
[b] Mean of two lots only.

latter eluted after zeinoxanthin from the alumina column, had an R_F value (0·50) lower than zeinoxanthin on the TLC plates and changed colour from yellow to blue-green on exposure to HCl gas. The absorption spectrum with maxima 20 nm lower than those of zeinoxanthin was consistent with a 5,8-epoxy carotenoid.

When the general properties are compared (Table 3), the most obvious difference lies in the vitamin C content (98 mg/100 g for IAC-4 guavas and 9–52 mg/100 g for the northeastern fruits). The fact that the IAC-4 guavas were freshly harvested while the northeastern fruits were bought from the market with no definite information on the date of collection must have some influence. The difference was so great, however, that varietal effects must have been at play. The large disparity in vitamin C levels had already been observed in the literature. For three Venezuelan guava cultivars, for example, values of 26·7, 85·8 and 214 mg/100 g were obtained (Rivas, 1969). The INCAP-ICNND gave a value of 218 mg/100 g. For Brasilian guavas, vitamin C contents of 54 to 560 mg/100 g had been reported (Moura Campos, 1958). These results have serious implications since guava is considered to be one of the richest sources of vitamin C, superior to oranges.

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REFERENCES

AOAC (1980). *Official methods of analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC.

Bauernfeind, J. C. (1972). Carotenoid vitamin A precursors and analogs in foods and feeds. *J. Agr. Food Chem.*, **20**, 456–73.

Fonseca, H., Nogueira, J. N. & Marcondes, A. M. S. (1969). Teor de ácido ascórbico e β-caroteno em frutas e hortaliças brasileiras. *Archv. Latinoamer. Nutr.*, **19**, 9–16.

Holdsworth, S. D. (1979). Fruits. In: *Effects of heating on foodstuffs.* (Priestley, R. J. (Ed)), Applied Science Publishers Ltd, London, 255.

INCAP-ICNND (1961). *Food composition Table for use in Latin America.* Institute of Nutrition of Central America and Panama, Guatemala. 54.

Klaui, H. & Bauernfeind, J. C. (1981). Carotenoids as Food Color. In: *Carotenoids as colorants and vitamin A precursors.* (Bauernfeind, J. C. (Ed)), Academic Press, New York. 48–317.

Moura Campos, F. A. (1958). Valor nutritivo de algumas frutas nacionais. II. A goiaba. *Arg. Bras. Nutr.*, **14**, 145–7.

Nakasone, H. Y., Brekke, J. E. & Cavaleto, C. G. (1976). *Fruit and yield evaluation of ten clones of guava* (Psidium guajava *L.*). Research Report, Hawaii Agricultural Experiment Station, 218, 17 pp.

NAS-NRC (1980). *Recommended dietary allowances* (9th edn). National Academy of Sciences, Washington, DC 55–60.

Nogueira, J. N., Sobrinho, J. S., Vincosvsky, R. & Fonseca, H. (1978). Efeito do armazenamento nos teores de ácido ascórbico e o β-caroteno em goiaba (*Psidium guajava* L.) liofilizada. *Archv. Latinoamer. Nutr.*, **28**, 363–77.

NRC (1982). *Diet, nutrition and cancer.* National Academic Press, Washington, DC.

Padula, M. & Rodriguez-Amaya, D. (1983). Comparison of the carotenoid composition and general properties of the processed juice of guava cultivar IAC-4 and commercial juices. *Ciênc. Tecnol. Alim.*, **3**, 109–16.

Rivas, N. (1969). Estudio das variedades de guayaba. Fac. Agron. Univ. Central de Venezuela, 1964. Unpublished. Cited in Czynrinciw, N., Tropical fruit technology. In: *Advances Food Research.* (Chichester, C. O. (Ed)), Academic Press, New York, **17**, 153.

Rodriguez, D. B., Raymundo, L. C., Lee, T. C., Simpson, K. L. & Chichester, C. O. (1976). Carotenoid pigment changes in ripening. *Momordica charantia* fruits. *Ann. Bot.*, **40**, 615–24.

Wenkam, N. S. & Miller, C. D. (1965). Composition of Hawaii fruits. *Bull.* 135, Univ. of Hawaii.