ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición
Vol. 45 N° 4, 1995

Carotenoid composition and vitamin A value of Brazilian loquat (*Eriobotrya japonica Lindl.*)

Helena T. Godoy [1], *and Delia B. Rodriguez Amaya* [2]

Departamento de Ciencia de Alimentos, Faculdade de Engenharia de Alimentos,
Universidade Estadual de Campinas, SP., Brasil.

SUMMARY. Six sample lots of loquat commercialized in Campinas, Brazil were analyzed for their carotenoid composition ß-carotene (7.8 µg/g), ζ-carotene (0.1 µg/g), neurosporene (1.1 µg/g), ß-cryptoxanthin (4.8 µg/g), 5,6-monoepoxy-ß-cryptoxanthin (0.6 µg/g), violaxanthin (1.6 µg/g), neoxanthin (0.8 µg/g) and auroxanthin (0.9 µg/g) were identified ß-Carotene and ß-cryptoxanthin were the principal pigments, being responsible for 44% and 27%, respectively, of the total carotenoid content (17.6 µg/g). Both were also the principal contributors to the vitamin A value of 175 RE/100g. The carotenoid composition of the Brazilian loquat resembles that of the Japanese loquat variety Tanaka.

RESUMEN. Contenido de carotenoides y vitamina A en níspero brasileño. Se analizó el contenido de carotenoides en seis lotes de muestras de níspero comercializadas en Campinas, Brasil. Se identificaron el ß-caroteno (7.8 µg/g), ζ-caroteno (0.1 µg/g), neurosporeno (1.1 µg/g), ß-criptoxantina (4.8 µg/g), 5,6-monoepoxy-ß-criptoxantina (0,6 µg/g), violaxantina (1,6 µg/g), neoxantina (0,8 µg/g) y auroxantina (0,9 µg/g). El ß-caroteno y la ß-criptoxantina fueron los principales pigmentos, siendo responsables por 44 y 27%, respectivamente, del contenido total de carotenoides (17,6 µg/g). Ambos fueron también los principales contribuyentes para el valor de vitamina A de 175 ER/100 g. La composición en carotenoides del níspero brasileño se asemeja al del níspero japonés variedad de Tanaka.

INTRODUCTION

A native of China, loquat (*Eriobotrya japonica (Thumb.) Lindl.*) is a yellow orange to golden orange sub-tropical fruit. Also known as Japanese plum, it has been grown for years in that country and is also cultivated in Israel and other subtropical regions. The plant belong to the family Rosaceae, subfamily Maloideae. The fruits appear in clusters, are ovoid or pyriform, about 4 cm long and contain a few large ovoid brown seeds (1).

Although the carotenoid composition of loquat had already been determined in Israel (2) and Japan (3,4), the inherent variability of food samples as a function of factors, such as varietal differences and geographic or climatic effects, justifies the analysis of loquats produced in other countries.

In Brazil, loquat has acquired increasing economic importance in recent years. Its harvest season coincides with the period (May to October) in which other fresh fruits are scarce in the domestic market. Brazilian loquat has also found its way into the international market, being one of the fresh fruits exported by the country. According to Ojima and Kigitano (5) the major varieties are «Mizuho», which is a cross between the Japanese varieties Kusunoki and Tanaka, and «Precoce», which is a cross between Tanaka and Mogi

Carotenids have gained renewed interest in recent years. In terms of their nutritional and technological importance, the emphasis has been for many years on the provitamins and the major pigments responsible for food color. Recent studies showing that the carotenoids, vitamin A active or not, may perform other important physiological functions, such as inhibition of cancer (6-10), immunoenhancement (11) and prevention of cardiovascular disease (12) demonstrate the importance of determining the complete composition.

1 Assistant Professor, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas (UNICAMP), P.O. Box 6121, Campinas, SP, Brasil.

2 Full Professor, Faculdade de Engenharia de Alimentos. Universidade Estadual de Campinas (UNICAMP), P.O. Box 6121, Campinas, SP. Brasil.

MATERIALS AND METHODS

Samples: The samples were obtained from supermarkets and the farmer´s market in the Campinas region. It was not possible to establish the variety, but it was probably Mizuho and/or Precoce, the most produced varieties. The fruits were medium sized, almost 50 g each, oval, yellow-orange, with easily removed peel, firm pulp, sweet to slightly acid taste. Lots of approximately 500 g were collecteed during the season. The fruits of each lot were peeled and homogenized, 150 g pulp was taken for analysis.

Analytical methodology: The carotenoid composition was determined as described in detail previously (13), with some modifications to make it more suitable to the sample being analysed. The pigments were extracted in a Waring blender with cold acetone, $MgCO_3$ and celite and filtered through a Buchner funnel, extraction and filtration being repeated until the residue turned colorless. The acetone extracts were combined and the carotenoids transferred to petroleum ether in a separatory funnel with the addition of water. The epiphase was washed 4 or 5 times with water to get rid of residual acetone. Saponification was undertaken with equal volume of 10% KOH in methanol with approximately 0.1g of BHT, at room temperature overnight. After washing and concentration in a rotary evaporater, initial separation was accomplished in a Mg0: Hyflosupercel (1:2) column developed with 4% diethyl eter and increasing concentration of acetone (10, 15 and 20%) in petroleum ether.

The much higher amounts of ß-carotene and ß-cryptoxanthin made the separation more difficult. The first two fractions were rechromatographed on neutral alumina columns (act II or III) to separate ß-carotene and ζ-carotene of the first fraction and neurosporene, ß-cryptoxanthin and 5,6-monoepoxy-ß-cryptoxanthin of the second fraction . The epoxides violaxanthin, auroxanthin and neoxanthin (fraction 3) could only be separated completely on silica gel plates developed with 20% acetone in petroleum ether. The ß-carotene and ß-cryptoxanthin fractions were submitted to rechromatography on $Ca(OH)_2$ (Mallinkrodt) column to verify the presence of *cis* isomers.

Identification was based on chromatographic behavior on column and TLC, visible absorption spectra and specific chemical reactions (epoxide test, cis-trans isomerization, acetylation and methylation) (14). Quantitation was based on the maximum absorbance as described by Davies (14).

The vitamin A value was calculated according to the NAS-NRC (15) ratio of 6 µg ß-carotene and 12 µg of the other provitamins to 1 retinol equivalent (RE).

Necessary precautions were taken to prevent degradation or alteration of pigments during analysis (e.g protection from light and high temperature, short analysis time).

RESULTS AND DISCUSSION

Confirmation of the identity of the carotenoids: Eight carotenoids were detected in the brazilian loquat. The carotenes, ß-carotene (λmáx 477, 450 and shoulder at 424 nm), ζ-carotene (λmáx 424, 400, 380 nm) (and neurosporene (λmáx. 472, 442, 420 nm), were identified through their typical absorption spectra in petroleum ether. The absense of substituents, already indicated by the order of elution from the column, was confirmed on the silica gel plates developed with 3% methanol in benzene where the carotenes run with the solvent front.

The xanthophylls, ß-cryptoxanthin (λmáx. 478, 450, and shoulder at 425 nm), 5,6-monoepoxy-ß-cryptoxanthin (λmáx. 472, 444, 420 nm), violaxanthin (λmáx. 470, 445, 420 nm) auroxanthin (λmáx. 428, 405, 384 nm) and neoxanthin (λmáx. 468, 438, 422 nm), presented absorption spectra compatible with their chromophores. The presence of hydroxyl groups was first indicated by the Rf values on the silical gel plates (0.49, 0.48, 0.15, 0.09 and origin, respectively) and confirmed by the positive response to acetylation with acetic anhydride. Reaction to methylation with acidified methanol, however, was negative, demonstrating that the hydroxy substituents were not located in allylic positions. The occurrence of epoxide groups was first shown by the transformation of the yellow colour to blue or green on exposure of the silica plates to HCl vapor. Its location at the 5,6-position in neoxanthin and 5,6- and 5´, 6´- positions in violaxanthin was confirmed by a 20 nm and 40 nm hypsochromic shift, respectively, on addition of 0.1N HCl to the pigment dissolved in ethanol. The presence of furanoid groups at the 5,8- and 5´, 8´- positions in auroxanthin was shown by the absorption spectrum with maxima already 40 nm lower than ß-carotene. *Cis*- isomers of ß-carotene and ß-cryptoxanthin were not detected.

Quantitative Composition: Table 1 shows thee quantitative composition ß-Carotene (7.8 µg/g) was the principal pigment and ß-cryptoxanthin (4.8 µg/g) appeared to be the second major carotenoid. The mean total carotenoid content was 17.6 µg/g and the vitamin A value was 175 RE/100 g or 1750 IU/ 100 g. The provitamins A were ß-carotene, ß-cryptoxanthin and 5,6-monoepoxy-ß-cryptoxanthin.

The carotenoid composition is presented in percentages in Table 2 so as to permit comparison with the data for Israeli Golden Nugget (2) and Japanese Mogi and Tanaka loquats (3). The Israeli data was given in percentages while the Japanese results were expressed in both µg/100g and percentages. In both studies, the number of sample lots analysed was not specified and no idea of between-sample variation was given.

TABLA 1
CAROTENOID COMPOSITION OF THE BRAZILIAN LOQUAT

Carotenoid	Concentration (μg/g fresh weight)[a] Range	Mean	SD
ß-carotene	7.4 - 8.0	7.8	0.3
ζ-carotene	0.1 - 0.2	0.1	0.1
neurosporene	0.9 - 1.5	1.1	0.3
ß-cryptoxanthin	4.6 - 5.0	4.8	0.1
5,6-monoepoxy-ß-cryptoxanthin	0.3 - 0.9	0.6	0.2
violaxanthin	1.5 - 1.7	1.6	0.1
auroxanthin[c]	0.8 - 1.0	0.9	0.1
neoxanthin	0.6 - 0.9	0.8	0.1
Total	16.9 - 18.1	17.6	0.2
Vitamina A value (RE/100 g)	166 - 189	175	12

a Ranges, means and standard deviations of six sample lots analyzed individually.
b Value calculated on the basis of the B-cryptoxanthin absorption coefficient.
c Value calculated on the basis of the B-carotene absoption coefficient. The carotenoids were in trans- configuration.

TABLE 2
COMPARISON OF THE CAROTENOID RELATIVE PERCENTAGENS IN BRAZILIAN, ISRAELI AND JAPANESE LOQUAT

Carotenoid	% of total Carotenoid Brazilian	Israeli[a] Var. Golden Nugget	Japanese[b] Var. Mogi	Var. Tanaka
cis-neo-ß-carotene	—	5.0	—	—
ß-carotene	44.6	33.0	29.7	41.9
ζ-carotene	0.7	—	1.0	2.5
γ carotene	—	6.1	—	—
neurosporene	6.2	—	—	—
mutatochrome	—	1.4	0.1	—
5,6-monoepoxy-ß-cryptoxanthin	3.5	2.8	—	—
5,6,5', 6'-diepoxy-ß-cryptoxanthin	—	2.2	13.0	6.6
ß-cryptoxanthin	27.3	22.0	32.8	30.9
cryptoflavin	—	1.6	—	—
lutein	—	3.2	—	—
cis-lutein	—	0.7	—	—
isolutein	—	1.7	—	—
violaxanthin	8,8	2,9	8,2	7,6
crysantemaxanthin	—	0.9	—	—
luteoxanthin	—	1.4	—	2.4
neochrome	—	1.0	—	0.4
neoxanthin	4.3	4.9	5.9	1.7
auroxanthin	4.9	—	—	0.4
Total carotenoid content (μg/g)	17.6	22.0	16.8	20.8
Vitamina A (RE/100g)	175	210	136	180

a Gross J, Gabai M. & Lifshitz A. (2) also reported 0.5% unknown (a+b), 5.5% carbonyl, 1.6% ß-carotene-like, 0.9% cryptoxanthin epoxyfuranoid and 0.2% neoxanthin-like. The authors also quantified phytofluene (0.5%).
b Kobayashi K., Iso H., Nishiyama K. & Akuts S. (3) also reported 5,6,5',8' -diepoxy-ß cryptoxanthin, an unknown and phytofluene and 6 more pigments present at percentages lower htan 0.1%.

There was excellent agreement between the Brazilian and Japanese (variety Tanaka) loquats especially in terms of ß-carotene (7.8 vs 7.0 μg/g), ß-cryptoxanthin (4.8 vs 5.2 μg/g) and violaxanthin (1.6 vs 1.3 μg/g). This is to be expected since, as mentioned earlier, the seeds of the loquats grown in Brazil come from Japan and are probably of the Tanaka or a cross with the Tanaka variety. Geographic effects appeared to be not important. The Brazilian fruit also contained neurosporene while the Japanese loquat had 5,6,5´, 6´ - diepoxy-ß-cryptoxanthin instead.

The Israeli loquat presented a somewhat different pattern, although ß-carotene and ß-cryptoxanthin were also the major carotenoids. Notably 6.1% γ-carotene and 3.2% lutein were found and, in addition, a series of epoxides and cis-neo-ß-carotene.

The cis-neo-ß-carotene (5%) detected in the Israeli loquat was not reported in the Japanese fruit. In Brazilian loquat a trace of 13-cis-ß-carotene was found only in one sample.

The Japanese workers found the epoxides mutatochrome, cryptoflavin, auroxanthin, cryptochrome and diol or triol 5,8-monoepoxides only in the canned loquat fruit stored for 6 months (16).

The total carotenoid content and vitamin A value of the Brazilian fruit (17.6 μg/g and 175 RE/100 g) were similar to the Japanese Tanaka variety (20.8 μg/g and 180 RE/100 g) and slightly lower than the Israeli Golden Nugget loquat (22.0 μg/g and 210 RE/100 g). This is reflected in the color of the fruit which is golden orange for the Israeli loquat and yellow-orange for the Brazilian fruit.

A more recent Japanese paper (4) reported much higher ß-carotene (21.1 μg/g) and ß-cryptoxanthin (9.6 μg/g) contents but no violaxanthin for the Tanaka variety (yellow-orange) The total carotenoid content (38.5 μg/g) was even higher than that of the Golden Nugget Israeli loquat. The loquat variety Toi was also analyzed giving the following composition in μg/g: ß-carotene (0.8), ß-cryptoxanthin (1.2), cryptoflavin (0.1), lutein (0.1), antheraxanthin (trace), luteoxanthin (0.1) and neochrome (0.2). The total carotenoid content was only 2.39 μg/g.

ACKNOWLEDGMENT

The autors wish to thank FAPESP (Fundaçao de Amparo a Pesquisa do Estado de São Paulo) for the financial support.

REFERENCES

1. Gross, J. Carotenoids, In: Pigments in Fruits, London, Academic Press, p. 146-17. 1971,
2. Gross, J. Gabai, M & Lifshitz, A. Carotenoids of *Eriobotrya japonica*. Phytochemistry, 12: 1775-1772, 1973.
3. Kobayashi, , Iso, H, Nishiyama, K & Akuta, S. Studies on carotenoid pigments and colour of fruits in Japan. Composition of carotenoids in loquat fruits (*Eriobotrya japonica Lindl.*). Jap Soc Food Sci Technol, 25: 191-195, 1978a.
4. Kon, M & Shimba, K. Cultivar difference of carotenoids in

loquat fruits. J Jap Soc Food Sci Technol, 35: 423-429, 1988.

5. Ojima, M & Kigitano, O. Cultura da nespereira. Rev Agric, 21: 1-7, 1978.
6. Colditz, GA, Lipnick, RJ, Millett, WC, Rosner, B, Posner, BM & Hennekens, CH, Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. Am J Clin Nutr, 41: 32-36, 985.
7. Krinsky, NI. Carotenoids in medicine. In: Carotenoids: Chemistry and Biology, Krinsky, NI, Mathews-Roth, MM & Taylor, RF (Eds). New York, Plenum Press, p. 279-291, 1990.
8. Mathews-Roth, MM. Carotenoids and cancer prevention experimental and epidemiological studies. Pure Appl Chem, 57 : 717-722, 1985.
9. Mathews-Roth, MM. Recent progress in the medical application of carotenoids. Pure Appl Chem, 63: 147-156, 1991.
10. Olson, JA. Biological actions of carotenoids. J Nutr 119: 94-95, 198.
11. Bendich, A. Carotenoids and the immune system. In: Carotenoids: Chemistry and Biology, Krinsky, NI, Mathews-Roth, MM/ Taylor, RF (Eds). New York, Plenum Press, p. 323-335, 1990.
12. Guster, H. Potential role of beta-carotene in the prevention of cardiovascular disease. Inter J Vit Nutr. Rer, 61: 277-291, 1991.
13. Rodríguez, DB, Raymundo, LC, Lee, TC, Simpson, KL & Chichester, CO. Carotenoid pigment changes in ripening *Momordica charantia* fruits. Ann Bot, 40: 615-624, 1976.
14. Davies, BH. Carotenoids, In: Chemistry and Biochemistry of Plant Pigments, 2 nd ed, vol. 2, Goodwin, TW (Ed). London, Academic Press, p. 38-65, 1976.
15. NAS-NCR. Recommended Dietary Allowances. 10th ed., Washington, DC, National Academy of Sciences, p. 78-92, 1989.
16. Kobayashi, K, Iso, H, Nishiyama, K & Akuta, S. The relationship between carotenoid pigments and colors of fresh and canned loquat fruits (*Eribotrya japonica Lindl.*). J. Jap Soc Food Sci Technol, 25: 426-430, 1978b.

Recibido: 10-10-1994

Aceptado: 25-08-1995