MULTICIENCIAS, Vol. 8, Nº 3, 2008 (267 - 271)
ISSN 1317-2255 / Dep. legal pp. 200002FA828

-Carotene in mango (*Mangifera indica L.*)

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Abstract

In this work, -carotene from two mango varieties: Criollo (Pico de Loro) and Haden, were standardized. The -carotene was extracted, separated, and identified by HPLC with a photodiode array detector (PDA) using a μ-Bondapak C_{18} 3.9 mm i. d. x 300 mm column in reverse phase and a non-aqueous isocratic system, constituted by 70:20:10 (AcCN / CH_2Cl_2 / MeOH) acetonitrile / dichloromethane / methanol. The chromatogram was resolved in 12 min, at a optima of 456 nm. The identification of -carotene was also corroborated by FTIR. The quantitative composition of beta-carotene measured as mg -carotene/kg pulp was determined by means of a calibration curve using -carotene and -apo-8-carotenal as standards. Average beta-carotene concentrations were 9.6±0.06 and 25.09±0.05 mg -carotene/kg pulp for the Haden and Criollo varieties, respectively. On the basis of beta-carotene content and the availability of fruit, mango can be considered as a potential raw material for -carotene extraction; it can be used as an extract to make an additive or as an antioxidant.

Key words: Mango fruit, -carotene, antioxidant, HPLC, FTIR.

Recibido: 11-06-2008 / Aceptado: 20-11-2008

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Resumen

En esta investigación, el β-caroteno en dos variedades de mango, el Criollo (Pico de Loro) y el Haden, fue estandardizado. El b-caroteno fue extraído, separado e identificado por HPLC con un detector de matriz de fotodiodos utilizando un μ-Bondapak C18 3.9 mm i. d. x 300 mm columna en fase reversa y un sistema isocrático no-acuoso, constituido por 70:20:10 (AcCN / CH2Cl2 / MeOH) acetonitrilo / diclorometano/ metanol. Se resolvió el cromatograma en 12 min, a una óptima lll de 456 nm. También, se corroboró la identificación del β-caroteno por FTIR. Se determinó la composición cuantitativa de beta-caroteno medida como β-caroteno/kg de pulpa por medio de una curva de calibrado usando β-caroteno y β-apo-8-carotenal como normas. La concentraciones promediadas de beta-caroteno eran 9,6 ± 0,06 y 25,09 ± 0,05 mg β-caroteno/kg pulpa para las variedades Haden y Criollo, respectivamente. Basado en el contenido de beta-caroteno y la disponibilidad de la fruta, se puede considerar al mango como una materia prima potencial para la extracción de β-caroteno; puede usarse como un extracto para hacer un aditivo o como un antioxidante.

Palabras clave mango (fruta), β-caroteno, antioxidante, HPLC, FTIR.

Introduction

Mango (*Mangífera indica* L.) is a fruit native from the south and southeast of Asia and the second most important tropical fruits in terms of production, marketing and consumer acceptance (Pott et al., 2003; Ribeiro et al., 2008). It is a rich source of antioxidants including ascorbic acid, carotenoids and phenolic compounds. The main producing country of this fruit is India and Brazil is one of the major mango exporting countries and has a great potential for expanding its market, since the climatic conditions allow cultivation throughout the year by the use of flower induction techniques (Ribeiro et al., 2008).

Mango has a characteristic yellow-orange color due to the presence of carotenoids, predominantly -carotene (pro-vitamin A), which makes the extraction of carotenoids from this fruit commercially important. -carotene yields two molecules of vitamin A. -carotene from fruit may enhance LDL degradation and prevent cardiovascular disease and lower the risk of various cancer disease because carotenoids are among the most effective singlet oxygen quenchers in nature (Shivashakara et al., 2004; Agostina et al., 2004; Chena et al., 2004; Marinova y Ribarova, 2007)

Mango fruit is very abundant in the Zulia State (Western Venezuela) and an excellent source of pro-vitamin A. Vitamin A consumed in Venezuela is imported, and is usually substituted by synthetic colorings which are harmful to the human health (Rivas, 1982).

Although the Open Column Chromatography (OCC) over magnesia, zinc carbonate, alumina, or silica is a common separation method for vitamin A, the development of HPLC has greatly increased the research on carotenoids since it has numerous advantages, such as versatility and sensitivity (Hussein et al., 1992). Besides, a better resolution of these structures and knowledge of the stereochemistry of these compounds have been possible thanks to development of powerful spectroscopic techniques coupled to the HPLC (Macrae, 1998; Acevedo, et al., 2004). The photodiode array detector (PDA) has made possible the use of relatively small spectral differences for the specific identification of carotenoids, adding these spectral data to those of their retention time (Scott, 1997).

In fact, Venezuela needs to produce this vitamin to reduce the amount of money exchange involved in importation and to use it as a food coloring in replacement of synthetic pigments, increasing the nutritional value of the foods.

The objective of this work was to extract the -carotene present in two varieties of mango and to characterize it by HPLC and FTIR.

Materials and Methods

Sample Selection: two mango varieties were selected: the variety Haden, which were supplied by Planimara, from "La Victoria" orchard, located in La Cañada, Urdaneta County of the Zulia State, and the variety Criollo (Pico de Loro), supplied by the "Roldanol" orchard, located in El Moján, Mara County of the Zulia State. Two different sample 50Kg lots of each variety were brought at the fully ripe stage in the laboratory.

Sample treatment: the mangoes were peeled and deseeded. The pulp was homogenized and 40 g were taken for immediate analysis. All the analyses were performed in the absence of light and under a nitrogen atmosphere in order to avoid the degradation of carotenoids.

Extract preparation and HPLC analysis: the carotenoids were carefully extracted with cold acetone, then transferred to a mixture of diethyl ether/ petroleum ether and saponified overnight at -10°C, using a 10% solution of methanolic potassium hydroxide, under a N_2 atmosphere; the saponified extracts were rinsed until they were alkali free, concentrated in a evaporator (T< 35°C) until dryness. 1mL of hexane and an appropriate aliquot of -apo-8 carotenal as external standard was added, evaporated under N_2 and redissolved in 1 mL of hexane.

Identification and quantification: the -carotene in the extract was identified and quantified by HPLC, using an equipment (Waters, Milford, USA) with the following modules: an automatic sampler (model 717E), a quaternary pump (model 600E) and photodiode array detector (PDA, model 996). The separation was carried out in a μ-Bondapak C_{18} column, 300 mm x 3.9 mm i. d., with an acetonitrile /dichloromethane/ methanol (70:20:10) mobile phase. The acquisition of the chromatograms and the analysis of the results were done with the Millenium[32] software. The equilibrium time at the initial conditions was 30 min. -carotene and -apo-8 carotenal (Sigma Chemical Co., St. Louis, USA) were used as standards and their calibration curves were used for the quantitative analysis by HPLC, calibration curves were made with four concentration in triplicates (Mercadante et al. 1997, Shivasshankara et al., 2004, Sanchez-Moreno et al., 2003).

The identification was confirmed by FTIR spectrometry, using a Perkin-Elmer FTIR 1600 spectrometer (Albuquerque et al., 2003).

Results and Discussion

The optimum (456nm) was determined by using -apo-8carotenal as the standard. Standardized conditions for HPLC analysis were an injection volume of 20 μL and a flow rate of 0.8 ml/min. The calibration curves for the chemical standards -carotene and -apo-8-carotenal presented lineal correlation coefficients of 0.9998 and 0.9896, respectively. It was obtained with the aid of the software Millenium[32] (Water Co.) -carotene was the major component present in the extract, and it indicates that the extract was properly resolved in 12 minutes. -carotene peak was identified by comparing the photo diode array spectrum at $_{max}$ with those in the literature (Bramley, 2007). The solvent system was chosen based on the work by Adewusi and Bradbury (1993), who suggested the use of an isocratic system of non-aqueous solvents to minimize the risks of solute precipitations and the preservation of the column lifetime.

The retention time of -carotene was 10.410 minutes for the mango Criollo and 10.414 min for the mango Haden. These retention times are lower than those reported by Cano and De Arcos (1994). The simplicity of the separation indicates that the extraction method applied was convenient to isolate the -carotene from the mango pulp.

Table 1. -carotene (mg -carotene/kg pulp) of mango by HPLC: Haden and Criollo (Pico de Loro).

Mango variety	Extract color	-carotene (mg -carotene/kg)[a]
Haden	Yellow	9.60 0.06
Criollo	Yellow-orange	25.09 0.05

[a]Mean standard deviation of 5 determinations.

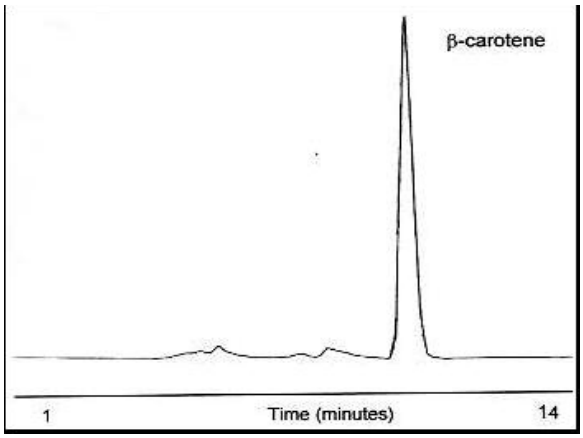

Figure 1. Reversed phase HPLC separation of -carotene in fresh mango Criollo using a μ-bondapack C_{18} stationary phase and 70:20:10 AcCN/CH_2Cl_2/MeOH mobile phase.

Mercadante et al. (1997) assured that sterols and xantophiles are common interferences in the determination of this type of compounds. The extraction method utilized in this work significantly eliminated this type of compounds from the sample (Fig. 1). Mango presented only one carotene by using a reverse phase chromatography, C_{18} column.

-carotene content of the extracts of the Criollo and Haden mangoes are shown in Table 1. The main component was -carotene as it has been reported by Cama and Jungalwala (1963), Cano and De Arcos (1994), Mercadante et al. (1997), Pott et al. (2003), and Chen et al., (2004).

The concentration of -carotene present in the Criollo variety was about three times higher than the concentration in the Haden variety. This result was expected due to the greater intensity of the yellow-orange color of mango Criollo. The content and variety of carotenoids in mango can be affected by many factors,i.e., growth condition, maturity and cultivar (Chen et al., 2004).

The -carotene content of the Criollo variety was higher than that reported for other mango varieties such as Keith, grown in Bahía, Brazil (15 mg -carotene/kg pulp), and Hilacha, grown in Maracaibo, Venezuela (12 mg -carotene/kg pulp), according to Rivas (1982). The content of -carotene in the Haden variety was lower than the content reported for other varieties. (Mercadante et al. 1997), found that fruit from hot climate had more -carotene content than those from moderate climate.

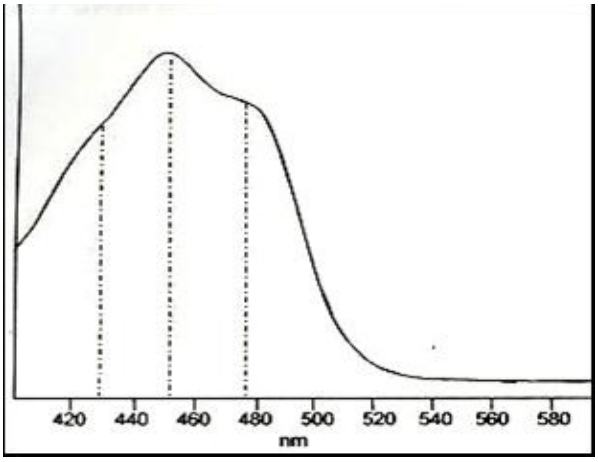

Figure 2. UV-Vis spectrum of -carotene extracted from fresh mango Criollo.

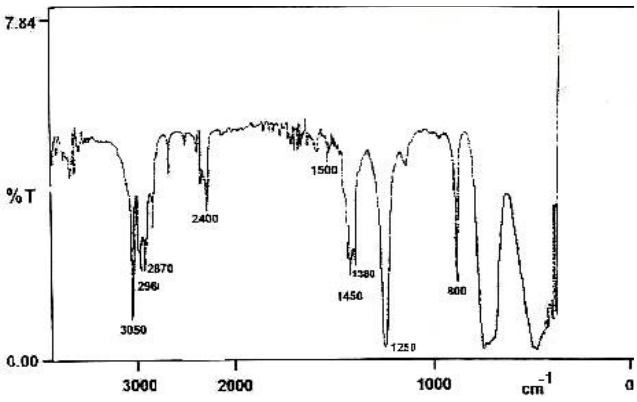

Figure 3. FTIR spectrum of -carotene extracted from fresh mango Criollo.

In this study the two varieties, both cultivated in hot climate (Zulia State), presented different amount of -carotene content among them.

The UV-VIS spectrum (Fig. 2) for the extract of the Criollo variety (similar to the Haden) showed the typical absorption wavelength of the -carotene, with three peaks (430.0nm, 453.9nm, and 477.0nm), one dull peak and the other two well defined. The lowest wavelength gets faded due to the number of double conjugated bonds (11 for provitamin A); it is necessary that the compound has at least 16 double conjugated bonds in order to observe one band of this type (Fennema et al. 2007).

FTIR spectrometry was used as an additional tool for the identification of the structure of the -carotene (Fig. 3). The FTIR spectrum of the -carotene extracted from the Criollo sample showed the intense absorption bands of the symmetrical and asymmetrical enlargements of the olefin C-H

bonds (3500 cm^{-1}), the characteristic moderately intense bands of symmetrical and asymmetrical enlargements of aliphatic C-H bonds (2920 cm^{-1} – 2860 cm^{-1}) of the methyl groups present in the molecule, and a band (2400 cm^{-1}) assigned to the absorption frequency characteristic of the CO_2. There is an irregular peak (1550 cm^{-1} – 1600 cm^{-1}) instead of the enlargement band of the bonds C = C due to the symmetry of the molecule (Silverstein et al., 1980).

There are also bands (1450 cm^{-1}- 1380 cm^{-1}) corresponding to symmetrical and asymmetrical flexions of the methyl groups mentioned before and a distinctive band of intense absorption (1250 cm^{-1}) characteristic of enlargements of typical C-O-C ether bonds; the presence of this band in the spectrum of the -carotene is due to traces of the solvent used in the extraction stage.

This work reveals that the Criollo mango variety is a good source of -carotene. It has a great potential to be used as a raw material for the production of beta-carotene.

Acknowledgment

The authors wish to thank the Universidad del Zulia and its Consejo de Desarrollo Científico y Humanístico (CONDES) for its financial support.

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