Carotenoid and Carotenoid Ester Composition in Mango Fruit As Influenced by Processing Method

M. Pilar Cano* and Begoña de Ancos

Department of Plant Products Science and Technology, Instituto del Frío, Ciudad Universitaria s/n, 28040 Madrid, Spain

The predominant carotenoids and carotenol fatty acid esters in extracts from fresh, frozen, and canned mango fruit slices have been separated and identified by high-perfomance liquid chromatography on two "in series" C_{18} reversed-phase columns using a diode array detector. The pigment pattern was composed of oxygenated carotenoids (xanthophylls), carotenol mono(fatty acid esters), hydrocarbon carotenoids, and carotenol bis(fatty acid esters). The major identified components were antheraxanthin, violaxanthin, *cis*-isomers, and epoxy derivatives of lutein and β-carotene. Canned mango fruit slices showed a significantly different pattern with β-carotene the main carotenoid component (90%). Large differences in Hunter color values were observed between fresh or frozen and canned mango slices. Hunter color values of frozen slices approached the color of fresh mangoes, excluding the higher values of L (lightness) for frozen product.

Keywords: *Carotenoids; carotenoid esters; mango fruit; effect of processing*

INTRODUCTION

Mango (*Mangifera indica* L.) is the second largest tropical crop next only to banana in terms of production and acreage. It is relished for its succulence, exotic flavor, and delicious taste (Subramanyam et al., 1975). However, changes in color, texture, and flavor of processed mango products have been studied. Heat is the principal agent in processing which alters thermolabile chemical and flavor ingredients of the mango (Doughtery, 1971; Varshney and Barhate, 1978). The mango slices may be canned in syrup and stored during long terms. The effects of different composition of the employed syrups on the quality of canned mango has been investigated by several researchers (Siddappa and Bhatia, 1955; Siddappa and Bathia, 1956; Avena and Luh, 1983). Mango varieties with lower reducing sugar content could be successfully converted to mango cereal flakes (Krishnamurthy and Siddappa, 1965). Canning mango puree is better than freezing or chemically preserving it, although freezing slows down chemical changes during low-temperature storage (Adsule and Roy, 1975). Frozen preservation of mango puree needs a previous enzymic heat inactivation to avoid the development of undesirable changes in flavor and color of the final product. However, when mango slices are frozen without thermal treatment, the thawed product does not show discoloration or off-flavors even after prolonged frozen storage (Marín et al., 1992).

Since carotenoids are among the most abundant micronutrients found in cancer-preventive foods, the determination of accurate qualitative and quantitative data on these classes of compounds in foods, particularly in fruits and vegetables, has become increasingly important. The development of HPLC techniques has made it possible to analyze and identify the carotenoid pattern of many yellow/orange fruits and vegetables such as apricots, peaches, cantaloupe, and pink grapefruit (Khachik et al., 1989; Beecher and Khachik, 1988; Chandler and Schwartz, 1987) or the chlorophyll/carotenoid pattern of green fruits and vegetables such as kiwi fruit, spinach, and green beans (Cano, 1991; Schwartz et al., 1981; Elbe et al., 1986).

Several researchers have investigated the carotenoid constituents in three stages of ripening of mango (John et al., 1970) or the changes in individual carotenoids on processing and storage of this fruit in slices or puree (Godoy and Rodriguez-Amaya, 1987). However, in these works the carotenoid pattern of the mango extracts were separated and analyzed by thin layer chromatographic techniques and traditional chromatographic methods. The carotenoid pattern reported by these authors consisted of more than 17 different carotenoids, with β-carotene the major carotenoid in the unripe and fully ripe mango. Epoxy carotenoids such as 5,6-monoepoxy-β-carotene, mutatochrome, *cis*-violaxanthin, luteotoxanthin, mutatoxanthin, and auroxanthin were observed in all three stages of ripening. Godoy and Rodriguez-Amaya (1987) reported that the changes in the carotenoid composition of processed mangoes are closely related to the mango cultivar. The carotenoid composition was practically maintained on processing mango slices, cultivar Tommy Atkins; the only significant change was the increase in luteoxanthin, compatible with the conversion of 5,6- to 5,8-epoxide. However, in cultivar Golden mango processed in puree, more evident transformations occurred, mainly a considerable loss of β-carotene (84%) after 24 months of storage.

In this paper, the extensive investigation of carotenoid patterns by high-perfomance liquid chromatography and the effects of the two most employed processing techniques to preserve mango slices, freezing and canning, have been carried out. HPLC conditions that separated as many as 33 carotenoids as well as several of their stereoisomers have been developed.

EXPERIMENTAL PROCEDURES

Reagents and Materials. Mangoes (*M. indica* L.) cv. Alphonso were obtained from commercial sources in unripe stage. Fruits were stored at 12 °C under 90% relative humidity until they were taken for analysis (ripe stage). Canned mango slices (cv. Alphonso) in syrup (17–19° Brix)

* Author to whom correspondence should be addressed [telephone 34-1-5492300 (ext. 29); fax 34-1-5493627].

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processed by conventional methods were supplied by Universal Food Co., Ltd., Bangkok (Thailand). For frozen product, firm ripe mango fruits (cv. Alphonso) were rinsed in tap water, drained and peeled. The cheeks of each fruit were cut off and sliced (approximate slice dimensions: 50 × 35 × 15 mm). The residual flesh was rejected because of the difficulty of obtaining uniform slices. Mango slices were frozen in an air-blast freezer operating at −40 °C, without any previous treatment. The slices were left in the freezer until their centers had reached −24 °C. Frozen slices were vacuum packed in plastic bags (Polyskin X12) containing 250 g of fruit and stored at −24 °C during 6 months until carotenoid analysis.

The reference samples of lutein and *all-trans*-β-carotene were provided by Hoffman-La Roche, Basel, Switzerland. HPLC grade solvents, methanol and ethyl acetate (Lab Scan, Ireland), were used without further purification. Tetrahydrofuran (THF, Panreac, Spain) used for extraction was stabilized with butylated hydroxytoluene (BHT; 0.01%).

Determination of Color Data. Triplicate samples (approximately 50 g) were pureed and placed in a 5-cm-diameter plastic dish to depth of not less than 2 cm. The sample dish was placed on the light port of a Hunter Lab Model M25-9 colorimeter. The instrument was standarized on a white plate and accuracy was checked with a standard yellow ($Y = 59.4$, $X = 57.5$, $Z = 43.1$). The sample dish was covered to avoid stray light. The L, a, and b values were recorded, and derived functions for hue (h), saturation (C), and total color differences (E) were calculated: $h = \arctan(b/a)$; $C = [(a^2 + b^2)^{1/2}$; $E = (L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$ (Von Elbe et al., 1986).

Pigment Analysis. Duplicates of all samples were extracted and analyzed for pigment composition by using high-perfomance liquid chromatography as follows.

Apparatus. A Hewlett-Packard Model 1050 quaternary solvent delivery system equipped with a Hewlett-Packard 1040A rapid-scanning UV−visible photodiode array detector was employed. The data were stored and processed by means of a Hewlett-Packard Model 9000/300 computing system and Color Pro plotter. The absorption spectra of the pigments were recorded between 300 and 600 nm at the rate of 12 spectra/min. The HP-9000 computer with a built-in integration program was used to evaluate the peak area and peak height. Absorption spectra of isolated components in various solvents were recorded on a Perkin-Elmer Lambda 15 UV−visible spectrophotometer.

Column. Separations were performed on two in series conected stainless-steel (10 cm × 4.6 mm i.d.) Hypersil ODS (5-μm spherical particles) columns (Hewlett-Packard), which were protected with a Hibar guard cartridge (3-cm length × 4.6-mm i.d.) packed with Spherisorp C_{18} (5-μm particle size).

Chromatographic Procedure. The analytical separations were carried out according to the procedure of Cano (1991). A gradient mixture of methanol/water (75:25), eluent A, and ethyl acetate, eluent B, was used, beginning at time 0 until time 10 (min) with a semifinal composition of eluent B (70%). The gradient eluent composition was followed at time 10 until time 20 (min) with the final composition of eluent B (100%). The flow rate employed was 1 mL/min, and the chromatographic runs were monitored at 440 nm. At the end of the gradient, the columns were reequilibrated to initial conditions by a new gradient, beginning at time 20 (min) until time 30 (min), with a final composition of eluent B (0%) at a flow rate of 1 mL/min.

Preparation of Fruit Samples for Extraction. Mango fruits were prepared for analysis in the same way they are prepared for consumption, i.e. inedible parts removed. The frozen samples were thawed in a refrigerator (2 h) until the pigment extraction, and the canned ones were drained before this operation.

Extraction. The extraction procedure was carried out according to a modified procedure of Bushway and Wilson (1982). Sodium sulfate and magnesium carbonate (200% and 10% of the weight of fruit, respectively) and tetrahydrofuran (100 mL) stabilized with BHT (0.01%) were added to each fruit sample (30 g) and homogenized. The extractions were carried out at 0 °C, cooling the blender jar in an external ice bath, in total darkness and under nitrogen atmosphere to prevent the degradation and the *trans*−*cis* rearrangements. The extract was filtered under suction, and the solid materials were washed several times with THF until the resulting filtrate was colorless. The combined THF extracts were concentrated on a rotary evaporator at 35 °C under nitrogen and were partitioned into dichloromethane and saltwater. The organic layer was washed with water (3 × 50 mL) to remove the water-soluble materials. The water layers were combined and washed with dichloromethane (3 × 50 mL) until dichloromethane extract was colorless. The organic layers were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in an appropiate volume (0.2 mL) of dichloromethane. Duplicate 25-μL samples were injected from each extract for HPLC analysis.

Saponification. Ethereal solutions of the fruit extracts (50 mL) were treated with methanolic potassium hydroxide (30%) (0.4 mL) under nitrogen atmosphere at room temperature for 2 h. The solution was partioned into a saturated aqueosus solution of sodium chloride and ether, and the organic layer was removed. The aqueous layer was washed several times with ether, and the organic layers were combined and washed several times with water until KOH was completely removed (pH 7.0). The solvent was evaporated and the residue dissolved in the appropriate solvent for chromatographic analyses.

Identification of Pigments. The pigment separation and identification are in agreement with those found previously (Cano, 1991; Marín, 1992) in kiwi fruits. Carotenoids were identified according to their chromatographic behavior on HPLC and UV−visible absorption spectra, by comparing both their retention time and the absorbance spectra with those of authentic carotenoids previously purchased or separated from fruits (Cano, 1991); also each peak maximum spectra was compared with those found in the literature (Köst, 1988). Pigment functional group examination was carried out by specific chemical tests: conversion of 5,6-epoxide group into 5,8-furanoid in acid medium, acetylation of free hydroxyl groups, and reduction with borohydride of ketone groups (Davies, 1976; Liaaen-Jensen, 1971).

RESULTS AND DISCUSSION

The major carotenoid constitutents separated from extracts of raw, frozen, and canned mango slices and their corresponding HPLC peaks in the order of elution on two in series C_{18} reversed-phase columns are shown in Table 1. In the present study, the class of chemical compounds can be classified as: (a) xanthophylls (oxygenated carotenoids); (b) carotenol mono(fatty acid esters); (c) hydrocarbon carotenoids (β-carotene and mutatochrome) and (d) carotenol bis(fatty acid esters). The HPLC chromatograms of the extracts from raw and frozen mango slices showed the same qualitative composition of carotenoids but significant differences in their quantitative distribution can be observed (Figures 1 and 2).

The employed chromatographic conditions can separate all carotenoid compounds within 17 min. Xanthophylls (peaks 1−16) are eluted under the first step of the proposed gradient, up to 70% of eluent B (ethyl acetate) at 10 min. A second gradient step, up to 100% of eluent B at 20 min, eluted the carotenol mono(fatty acid esters) (peaks 17 and 20−25), the only hydrocarbon carotenoid, β-carotene (peak 26) and, the oxygenated hydrocarbon, mutatochrome (*cis* and *trans*) (peaks 18 and 19), and the last carotenol bis(fatty acid esters) (peaks 27−33).

The chromatographic characterization and separation of the carotenol mono- and bis(fatty acid esters) from their corresponding carotenoids in extracts of several fruits and vegetables have been achieved when a considerable development of HPLC methodology was obtained (Khachik and Beecher, 1988; Khachik et al.,

Table 1. Identification of Peaks of Carotenoids of Raw, Frozen, and Canned Mango Extracts Separated by HPLC

peak	carotene	λ, nm
1	*trans*-violaxanthin	420, 442, 470
2	*trans*-luteoxanthin	396, 420, 448
3	9-*cis*-neoxanthin	412, 436, 464
4	violaxanthin	420, 442, 470
5	neochrome	398, 422, 444
6	lutein 5,6-epoxide	420, 440, 472
7	lutein epoxide	418, 440, 472
8	neolutein epoxide A	412, 436, 464
9	neolutein epoxide B or B′	420, 440, 470
10	flavoxanthin	398, 422, 448
11	mutatoxanthin	396, 418, 442
12	neolutein B	418, 442, 470
13	neolutein B′	420, 440, 470
14	neolutein A	412, 436, 464
15	*trans*-antheraxanthin	418, 440, 470
16	*cis*-antheraxanthin	412, 436, 464
17	violaxanthin monoester	420, 440, 466
18	*trans*-mutatochrome	404, 426, 454
19	*cis*-mutatochrome	(292), 404, 426, 454
20	violaxanthin monoester	416, 436, 466
21	violaxanthin monoester	414, 436, 464
22	neolutein B monoester	418, 440, 470
23	violaxanthin monoester	412, 436, 464
24	neolutein B monoester	418, 440, 470
25	violaxanthin monoester	412, 436, 464
26	β-carotene	428, 450, 478
27	neolutein B diester	420, 442, 470
28	neolutein B diester	418, 440, 470
29	violaxanthin diester	412, 436, 464
30	neolutein B diester	418, 440, 470
31	violaxanthin diester	412, 436, 464
32	neolutein B diester	418, 440, 470
33	violaxanthin diester	414, 436, 464

1988, 1989). Other authors studied the pigment composition of various cultivars of mango fruits found that in Alphonso variety (Jungalwala et al., 1963) as well as in Bandami variety (John et al., 1970) it is possible to separate by conventional liquid–solid chromatography and then to identify by spectroscopic and chromatographic behavior 14 or 15 different carotenoid compounds. These carotenoids were ascribed to two classes of compounds, xanthophylls and hydrocarbon carotenoids, and identified the major ones as β-carotene, phytofluene, phytoene, luteoxanthin, violaxanthin, anteraxanthin, mutatoxanthin, auroxanthin, and mutatochrome.

In the present work, HPLC chromatograms of the extracts of raw and frozen mango slices showed the same 33 different carotenoid compounds but in different proportions (Figures 1 and 2). β-Carotene was the major carotenoid compound in both extracts, and the only hydrocarbon carotenoid present in the sample extracts. In these chromatograms, 16 xanthophylls have been identified by comparison of their absorption maxima and HPLC retention times with the reported literature values. Table 1 shows the absorption maxima values of these compounds in the HPLC solvent. The major xanthophylls in raw and frozen mango extracts are *cis*-antheraxantin (λ_{max} = 436 nm) and *trans*-antheraxanthin (λ_{max} = 440 nm) (peaks 15 and 16). In addition, other xanthophylls as luteoxanthin, mutatoxanthin and violaxanthin may be observed in the same extracts. However, the identification of other oxygenated carotenoids, auroxanthin, zeaxanthin, and cryptoxanthin, reported by Jungalwala et al. (1963) in mango cv. Alphonso, was not possible by the employed HPLC method. Therefore, in our study, a good separation between *cis*- and *trans*-violaxanthins which show the same absorption maximum (λ_{max} = 442 nm) (peaks 1 and 4) was obtained. Thus, in these samples a *cis*-isomer of neoxanthin identified as 9′-*cis*-neoxanthin (peak 3, Figure 2). Peak 5, in raw and frozen mango chromatograms, showed retention time and absorption maxima (λ_{max} = 398, 420, 444 nm) characteristic of neochrome (*cis* and *trans*).

all-trans-Lutein was not present in these mango extracts, but three of its *cis*-isomers were identified by comparison of their UV–visible spectra with those found for neolutein B, B′, and A samples separated and identified in other fruits (Cano, 1991) and vegetables (Khachik, 1986). Neolutein B and neolutein B′ present small hypsochromic shifts (4–8 nm) in the absorption maxima with respect to that of *all-trans*-lutein and the absence of a strong cis peak in the near-UV region in the absorption spectra, suggesting the possibility of 9- and 9′-cis isomers. The visible absorption of neolutein A contained an intense cis peak in the near-UV region, being tentatively identified as 13- or 13′-*cis*-lutein. Neolutein B (λ_{max} = 442 nm) and neolutein A (λ_{max} = 436 nm), peaks 12 and 14, showed a good separation, being the second most abundant xanthophylls lower than *cis*- and *trans*-antheraxanthins, in raw mango extracts (Figure 1). Peak 13 was identified as neolutein B′ (λ_{max} = 442 nm). Although *all-trans*-lutein is the xanthophyll more common in fruits and vegetables, in our mango samples this pigment was only present in unripe mangoes, disappearing by transformation to epoxy derivatives through fruit ripening (John et al., 1970).

Peaks 6–9 were assigned to these epoxy derivatives of lutein and of its *cis*-isomers, due to their absorption maxima and retention times which are in agreement with the reported values for these compounds (Köst, 1988). Peak 6 was identified as lutein 5,6-epoxide (λ_{max} = 420, 440, and 472 nm) and peak 7 as lutein epoxide (λ_{max} = 418, 440, and 470 nm). Peaks 8 and 9 were identified as epoxy derivatives of neolutein A (λ_{max} = 412, 436, and 464 nm) and neolutein B (λ_{max} = 420, 440, and 470 nm), respectively.

The chromatographic profiles of the extracts obtained from raw and frozen mango slices show the presence of a variety of carotenoid compounds with longer retention times than xanthophylls in the employed two reversed-phase C_{18} columns. These compounds were identified as neolutein and violaxanthin mono(fatty acid esters) (Figures 1 and 2). The presence of carotenol mono(fatty acid esters) in fruits was reported by Khachik et al. (1989). These authors obtained the separation of these compounds by similar chromatographic conditions as reported in the present work. The carotenol fatty acid esters identification was carried out by comparison of their retention times and spectrophotometric behavior with real ester compounds obtained by synthesis from the isolated carotenols (Khachik et al., 1988).

In the carotenoid profile of raw and frozen mango extracts, two peaks, 22 and 24, could be attributed to two mono(fatty acid esters) of neolutein B, showing absorption maxima 418, 440, and 470 nm. Probably, peak 22 could be assigned to neolutein B myristate and peak 24 to neolutein B palmitate taking into account the reported elution order of carotenol mono(fatty acid esters) from a C_{18} reversed-phase column (Khachik and Beecher, 1988). In this way, *trans*-violaxanthin myristate and *trans*-violaxanthin palmitate were identified, peaks 17 (λ_{max} = 420, 440, and 466 nm) and 18 (λ_{max} = 416, 438, and 466 nm), respectively. Regarding the slight

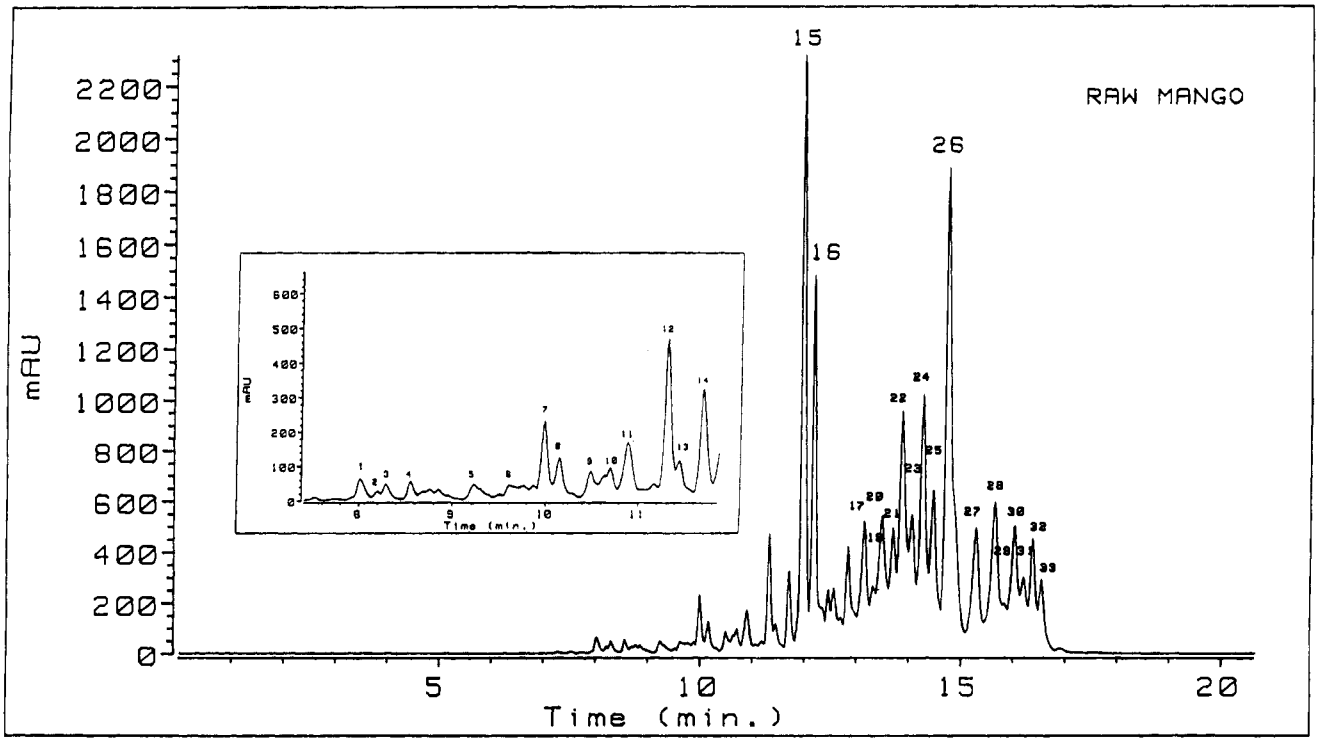

Figure 1. HPLC gradient profile of raw mango extract. Peak identifications are given in Table 1.

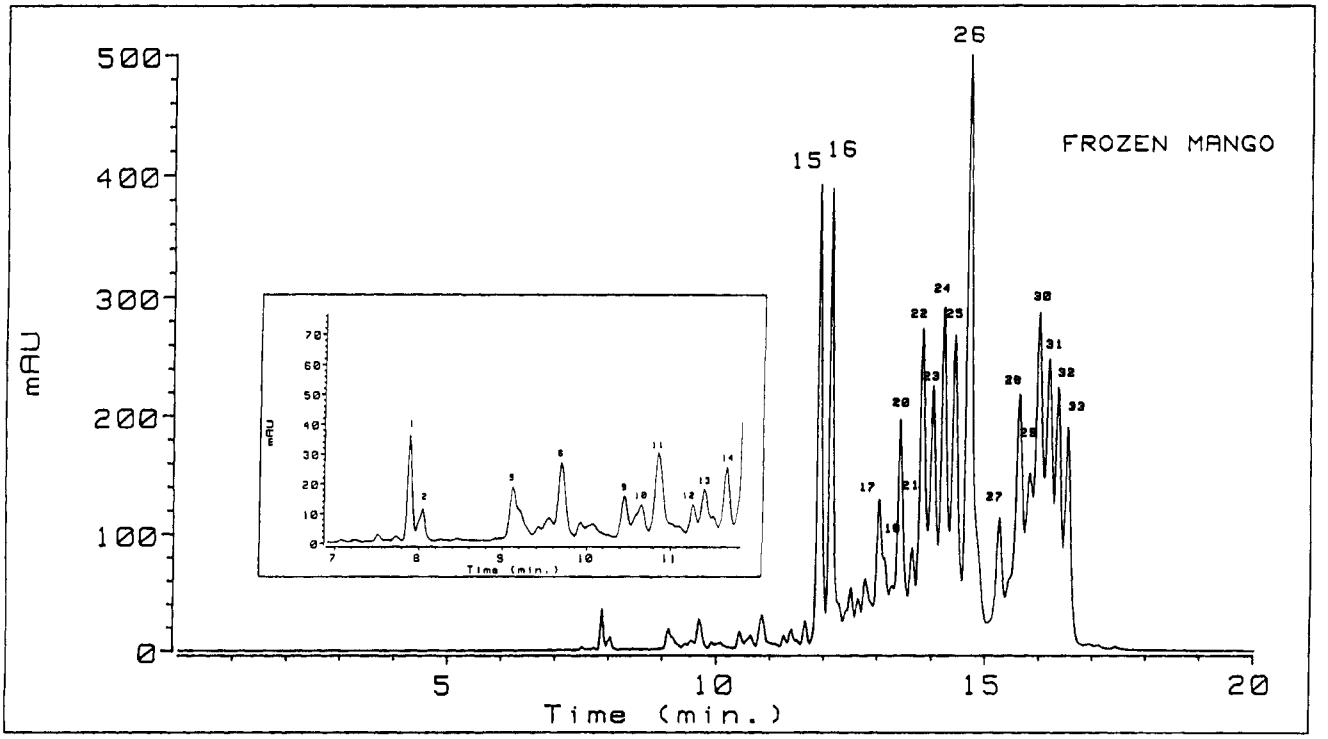

Figure 2. HPLC gradient profile of extracts obtained from frozen/thawed mango slices. Peak identifications are given in Table 1.

hypsochromic shift which show the absorption maxima of *cis*-carotenoids versus the *trans*-isomers ones, peaks 21, 23, and 25 (λ_{max} = 412, 436, and 464 nm) were attributed to mono(fatty acid esters) of violaxanthin.

Particularly noticeable is the presence of carotenol bis(fatty acid esters) in raw and frozen mango extracts, because these compounds were only reported to be present in peaches (Khachik et al., 1989). Peaks 27, 28, 30, and 32 (λ_{max} = 412, 436, and 470 nm) and peaks 29, 31, and 33 (λ_{max} = 414, 436, and 464 nm) were identified as bis(fatty acid esters) of neolutein and violaxanthin, respectively.

Saponification of raw mango extract produced an evident transformation of carotenoid profile (Figure 3; Table 2). Chromatogram obtained from this sample showed the disappearance of the peaks assigned to the carotenol mono- and bis(fatty acid esters) of neolutein and violaxanthin, observing the regeneration of some xanthophylls as violaxanthin, *trans*-violaxanthin, and the nonaltered presence of β-carotene. Although the

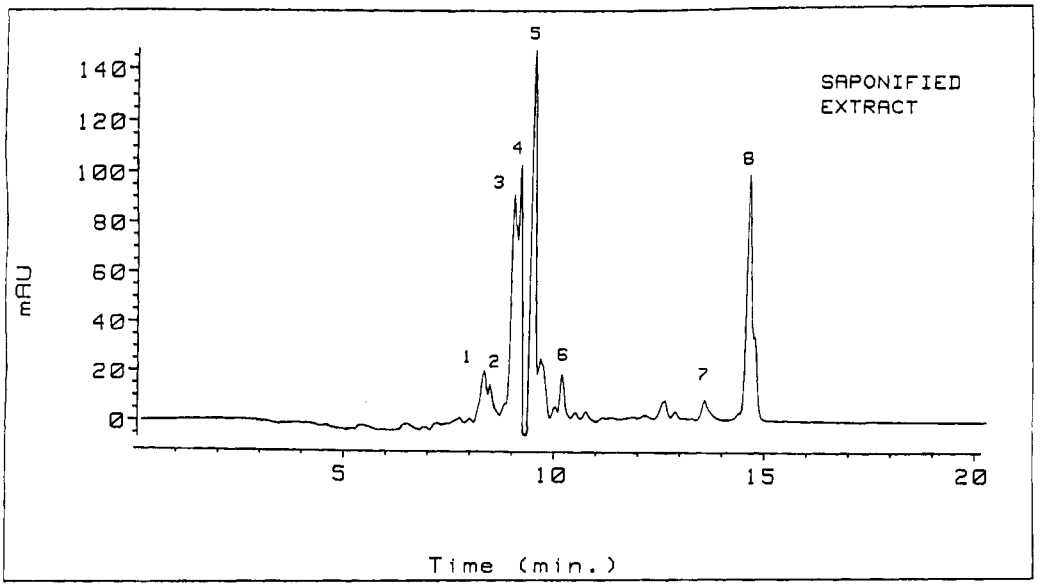

Figure 3. HPLC gradient profile of raw mango extract after saponification. Peak identifications are given in Table 2.

Table 2. Peak Identification of Carotenoids of Saponified Extract of Raw Mango

peak	carotene	λ_{max}, nm
1	*trans*-violaxanthin	420, 442, 470
2	*trans*-luteoxanthin	396, 420, 448
3	violaxanthin	420, 442, 470
4	neochrome	398, 422, 444
5	luteoxanthin	398, 418, 442
6	lutein	420, 446, 472
7	*trans*-mutatochrome	404, 426, 454
8	β-carotene	428, 450, 478

considerable loss of carotenoids, mainly xanthophylls, is a consequence of the saponification, this reaction is a good procedure to check the presence of carotenol fatty acid esters in plant extracts (Khachik et al., 1986).

The chromatogram of extracts from canned mango slices is shown in Figure 4. The carotenoid profile is significantly different from the one obtained for raw and frozen mango slices (Figures 1 and 2). Thermal treatment of the canning process produced the degradation of xanthophylls carotenoids. β-Carotene is the major carotenoid after the canning process being approximately 90% of the remaining total carotenoid concentration. In this chromatogram the presence of mutatochrome *cis* and *trans* can be observed (peaks 18 and 19), in the relative amounts of 5% and 7%, respectively. Peaks attributed to mono- or bis(fatty acid ester) of the carotenol compounds were not found, while the concentration of β-carotene did not suffer any significative loss by the employed processing method, freezing or canning. Mango slices canned by conventional methods in syrup, produced some important changes in the profile of the major carotenoids present in raw mango extracts. This resulted in changes of the nutritional value of the canned product. However, freezing preservation of mango slices gives a processed product with very similar appearance and color to the raw fruit. The carotenoid composition is comparable to the fresh product in its nutritional value.

Color of fresh, frozen, and canned mango slices in terms of Hunter color values is given in Table 3. There was little difference among fresh and frozen mango slices in terms of b parameter (yellowness), h (hue), and C (the calculated saturation value). Large differences

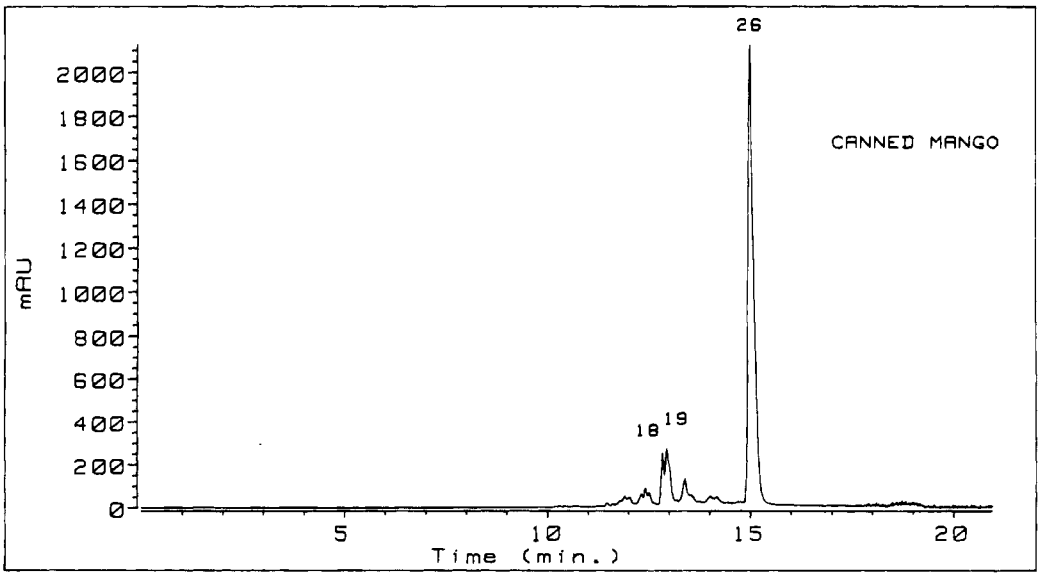

Figure 4. HPLC gradient profile of canned mango extract. Peak identifications are given in Table 1.

Table 3. Hunter Color Values[a] of Fresh, Frozen, and Canned Mango Slices

sample	L	a	b	h	C	E^b
fresh mango	48.43a	−1.05a	29.02a	87.92a	29.53a	
frozen mango	49.51b	−0.79b	29.40a	88.46a	29.41a	1.13a
canned mango	46.82c	2.45c	27.85b	84.46b	27.95b	4.02b

[a] Values in the same column with the same letter are not statistically different ($p = 0.05$). $h = \arctan(b/a)$ (hue); $C = (a^2 + b^2)^{1/2}$ (calculated saturation value); $E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$ (total color difference). [b] Determined from fresh ripe mango values.

were noted in the L (lightness) and a (greenness) values. Frozen mango slices turned more luminous and less green, maintaining their yellowness value. In this way, ΔE (total color difference) did not acquire large values, only 1.13 for the frozen product relative to the fresh one. However, this parameter was significantly different for the canned fruit. Total color difference value of 4.02 was obtained comparing the fresh to the canned. In terms of Hunter color parameters canned mango slices showed a dark orange color very different to the natural brigh yellow-orange of this mango variety.

Taking into consideration the HPLC studies of carotenoid composition of fresh, frozen, and canned samples, it is possible to conclude that conventional canning of mango slices in syrup produces important and dramatic changes in the color and pigment pattern. This results in a commercial product with a dark orange appearance different from that of the fresh fruit. The freezing process of mango slices in the assayed conditions led to a mango product with similar color characteristics and appearance to the fresh fruit with only slight changes in its pigment pattern.

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