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Contributed Paper

Carotenoid Composition and Antioxidant Activity of Extracts From Tropical Fruits

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ABSTRACT

The aim of this study was to determine the carotenoid composition, as well as the antioxidant activity of carotenoid extracts of fruits from Yucatan, Mexico. Among the fruits evaluated, the mamey sapote (36.41 mg of β -carotene/100 g of edible portion) obtained the highest total carotenoids content, while the red mombin, green sugar apple, green-yellow mombin, yellow cashew, purple sugar apple and green nance showed greater antioxidant activity of carotenoids extract using the ABTS assay. When the antioxidant activity of the carotenoid extracts was measured with the DPPH assay, the edible portion of the green nance was found to have the highest antioxidant activity. Four carotenoids (two xanthophyll's and two carotenes) were identified in the fruits. The mamoncillo, red cashew and yellow cashew obtained high values of retinol equivalents (1,266-2,908 μ g/100 g of edible portion). Lutein and β -cryptoxanthin contents showed a positive correlation with antioxidant activity by ABTS ($R=0.41$ and $R=0.31$, respectively). The results indicate a perspective for the exploitation and use of these tropical fruits in the diet, given their antioxidant activity with beneficial in human health.

Keywords: carotenoids, antioxidants, tropical fruits, antioxidant activity, lutein

1. INTRODUCTION

Carotenoids are lipophilic compounds, responsible for the red, orange and yellow hues of fruits and vegetables; they are also used as additives to confer a yellow-reddish

colour to many foods [1]. Approximately 750 carotenoids have been identified to date [2]. However, only a fraction of these compounds are absorbed and utilized by humans, and only

a small percentage serve as precursors of vitamin A. Carotenoids, which contain an unsubstituted β -ionone ring, including β -carotene, α -carotene, β -cryptoxanthin, and α -cryptoxanthin, have the ability to be converted into vitamin A *in vivo* [2]. The biological effects of vitamin A include growth promotion, cellular differentiation, immune function, embryonic development, and gap junction communication [3].

Several epidemiological studies have suggested that consumption of vegetables and fruits containing carotenoids helps prevent the development of degenerative diseases such as cardiovascular diseases, macular degeneration, some types of cancer and age-related cataracts [4]. The health benefits of carotenoids are most probably due to the antioxidant activities of their electron-rich conjugated system, both by quenching singlet oxygen and by scavenging radicals to terminate the chain reactions [5]. Such properties make these compounds ideal for the ever-increasing functional food industry while promoting the consumption of the natural products in which they are contained.

In tropical regions there is a great diversity of fruits that are consumed by wild animals, indigenous people or farmers. Many tropical fruits can be considered a reservoir of bioactive substances of special interest due to their possible health-promoting properties. Recently, Moo-Huchin *et al.* [6] reported that in Yucatan, Mexico, there are a large number of tropical fruits containing a considerable amount of phenolic compounds and antioxidant activity in the pulp with red and yellow tones such as the star apple, cashew, mombin, mamey sapote, white sapote, sugar apple, sapodilla, dragon fruit, nance, ilama, custard apple, mamoncillo and black sapote, which are of potential interest to agro-industry and constitute a possible source of income for the local population in the near future.

Some of these fruits are also cultivated and commercialized on a small scale in Brazil [7] and Panama [8]. The fruits cultivated in Yucatan, Mexico are characterized by their high content of water (71.6-87.5%), total soluble solids (8.95-22.3°Brix) and moderate acidity (0.19-1.87%); making them more attractive to consumers [6]. Taking into consideration the supporting information regarding the potential health benefits of carotenoids, the composition of carotenoids and their antioxidant activity in fruits collected in Yucatan needs to be investigated and reported. In this regard, the aim of this study was to determine the content of individual carotenoids and to demonstrate their antioxidant activity in tropical fruits grown in Yucatan, Mexico. To the best of our knowledge, this is the first paper presenting comprehensive data on individual carotenoids content and their antioxidant activity in tropical fruits from Yucatan, Mexico.

2. MATERIALS AND METHODS

2.1 Chemical Reagents

β -carotene, β -cryptoxanthin, lycopene, lutein, ABTS, DPPH and Trolox, were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). All other chemicals used were of analytical grade.

2.2 Samples

Nineteen fruits, which are commonly cultivated and consumed in Yucatan, Mexico, were chosen for this study (Table 1). About 5 kg of each fruit species (except sapodilla) were purchased at eating ripeness from the local markets in Yucatan, Mexico during 2012.

Five kg of sapodilla fruits were harvested from a single tree in an orchard located in the municipality of Cansahcab, in Yucatan, Mexico and were identified according to quality based on the stage of

physiological maturity and determined by the absence of latex. The fruits were stored at 25°C to reach an eating ripeness according to Moo-Huchin *et al.* [9].

The fruits without blemishes or damage were selected and sent to the laboratory for edible portion extraction. After the fruits

had been cleaned with tap water, the edible portion were extracted manually with a knife, processed in a blender and stored in sealed plastic bags. The carotenoid pigments were extracted from the fresh fruits on the same day of collection and when the sapodilla reached its eating ripeness.

Table 1. List of the tropical fruit species from Yucatan included in the study and parts analysed.

English name	Common name	Scientific name	Edible portion used
Green star apple	Caimito verde	<i>Chrysophyllum cainito</i> L.	Pulp
Purple star apple	Caimito morado		
Yellow cashew	Maranón amarillo	<i>Annacardium occidentale</i>	Pulp
Red cashew	Maranón rojo		
Green-yellow mombin	Ciruela verde-amarillo	<i>Spondias purpurea</i> L.	Pulp+peel
Red mombin	Ciruela roja		
Mamey sapote	Mamey	<i>Pouteria sapota</i> Jacq.	Pulp
White sapote	Zapote blanco	<i>Lucuma hypoglauca</i> Stanley	Pulp
Green sugar apple	Saramuyo verde	<i>Annona squamosa</i> L.	Pulp
Purple sugar apple	Saramuyo morado		
Sapodilla	Chicozapote	<i>Manilkara sapota</i> L.	Pulp
Dragon fruit	Pitahaya	<i>Hylocereus undatus</i> Haworth	Pulp+seed
Yellow nance	Nance amarillo	<i>Byrsonima crassifolia</i>	Pulp+peel
Green nance	Nance verde		
Red nance	Nance rojo		
Ilama	Anona	<i>Annona diversifolia</i>	Pulp
Custard apple	Anona roja	<i>Annona reticulata</i>	Pulp
Mamoncillo	Uaya	<i>Melicoccus bijugatus</i> (Jacq.)	Pulp
Black sapote	Zapote negro	<i>Diospyros digyna</i>	Pulp

2.3 Extraction of Carotenoids

Extraction of carotenoids was carried out according to the method developed by Chen *et al.* [10]. 10 g of edible portion of each fruit were placed in a vessel, protected from light, and mixed with 50 mL of extraction solvent (hexane/acetone/ethanol: 70:15:15, v/v/v). The mixture was stirred for 1 h using an orbital shaker. Afterwards, 5 mL of 40% KOH in methanolic solution

were added, and the solution was saponified at 25°C in the dark for 2 h. Subsequently, 30 mL of hexane were added, the mixture was shaken vigorously and the upper layer was collected. The lower layer was extracted twice and the supernatant was also collected and filtered through sodium sulphate powder to remove traces of water. The supernatant obtained was pooled and stored at -80°C under nitrogen atmosphere (99.9%

purity) in the dark until analysis.

2.4 Total Carotenoids

Total carotenoid content in the extracts was determined spectrophotometrically at 450 nm in a UV-Vis spectrophotometer PerkinElmer Lambda 11. A calibration curve (0-50 ppm) was prepared using β -carotene in hexane as the standard and hexane as the blank. The results were expressed as mg β -carotene/100 g of edible portion.

2.5 Antioxidant Activity Determinations

2.5.1 DPPH radical-scavenging assay

DPPH (2,2'-diphenyl-1-picrylhydrazyl) assay was conducted according to the Brand-Williams *et al.* [11] method with some modifications. The stock solution was prepared by mixing 2.5 mg of DPPH radical with 100 mL of methanol. The solution absorbance was adjusted at 0.7 ± 0.02 in 515 nm using an UV-Vis spectrophotometer PerkinElmer Lambda 11. 3.9 mL of DPPH radical were placed in a test tube and 100 mL of carotenoids extract or standard were added (methanol was used as blank). The decrease in absorbance at 515 nm was measured at 1 min intervals for the first 10 min, and then at 5 min intervals until stabilization. Based on a preliminary study, the time required to obtain DPPH readings of each fruit were as follows: 15 min (green nance); 10 min (red cashew and dragon fruit) and 5 min (green star apple, purple star apple, yellow cashew, green-yellow mombin, red mombin, mamey sapote, white sapote, green sugar apple, purple sugar apple, sapodilla, yellow nance, red nance, ilama, custard apple, mamoncillo and black sapote). Calibration curve was prepared using Trolox as standard and results are expressed as mM Trolox equivalents/100 g of edible portion.

2.5.2 ABTS radical-scavenging activity

The ABTS (2,2'-Azinobis-3-ethylbenzotiazoline-6-sulphonic acid) assay was conducted according to Miller *et al.* [12]. ABTS^{•+} cation was generated through the interaction of 19.2 mg of ABTS dissolved in 5 mL of HPLC-grade water and 88 mL of 0.0378 g/mL potassium persulfate ($K_2S_2O_8$). The cation was incubated in the dark at room temperature for 16 h. The ABTS activated radical was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 30 mL of carotenoids extract or standard to 2970 mL of diluted ABTS solution, absorbance was recorded 7 min after mixing. Calibration curve was prepared using Trolox as a standard and results are expressed as mM Trolox equivalents/100 g of edible portion.

2.6 HPLC Analysis of Individual Carotenoids

Identification and quantification of carotenoid pigments were carried out using HPLC-1220 Agilent equipped with a UV-Visible detector at 476 nm. Extracts were prepared as mentioned previously (Section 2.3) and were evaporated at 35°C using a rotary evaporator (Buchi R-205, Labortechnik, Switzerland). The residue was reconstituted in 1.5 mL of solvent (methanol/methylene chloride: 50:50, v/v) [13]. An aliquot was filtered through a 0.45 mm membrane and aliquots of 20 mL were injected in the HPLC system. A 250 × 4.6 mm i.d., 5 mm, Nucleosil C18 column was used (operated at 25°C). Mobile phase consisted of acetonitrile:1-butanol (7:3, v/v, A) and methylene chloride (B), at a flow rate of 1.0 mL/min. Elution gradient was 99% (A) and 1% (B) initially, increasing to 4% (B) in 20 min and then returned to 1% (B) in

22 min [13]. Identification of compounds was achieved by comparing the retention times and the spectra as well as by the addition of standards. The concentrations of pigments were calculated with the help of corresponding external standards.

2.6 Statistical Analysis

All extraction assays were carried out in triplicate and a duplicate of each extract was analysed. Results were expressed as means \pm standard deviation (SD). Analysis of variance (ANOVA) was carried out by Statgraphics Plus software, version 2.1 (Manugistic, Inc., Rockville, MD, USA). The comparison of means was performed by Tukey test. Statistical differences were considered to be significant ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Total Carotenoids Content in Tropical Fruits

Some of the fruits selected for the present study are unknown outside their natural range, some are barely known and some are well known and marketed in Mexico.

The important role of plant-derived carotenoids in the prevention of a number of human health problems such as certain types of cancer, heart disease and age-related macular degeneration [14] has motivated the interest in studying these pigments found in tropical fruits grown in Yucatan, Mexico. The tropical climate from Yucatan favors the occurrence of a wide variety of carotenogenic fruits. Normally, fruits are exposed to sunlight during their growth. Sunlight favours the breakdown of chlorophyll and induction of carotenogenesis.

In this work, the total carotenoids content in the edible portion of a group of tropical fruits grown in Yucatan, Mexico was determined (Table 2). According to the

results obtained in this study, the total carotenoids content of the 19 fruits under study, expressed as mg of β -carotene/100 g of edible portion varied between 0.70 and 36.41 mg/100 g. Among the fruits evaluated, the mamey sapote presented the highest total carotenoids content, while the green sugar apple obtained the lowest amount of this compound. These results indicate that the mamey sapote is the most important source of total carotenoids. The total carotenoids content reported for the mamey sapote was higher than those reported for 14 non-traditional fruits of Malaysia (values ranging from 1.41 to 19.83 mg of β -carotene/100 g of edible portion) [15] and for 12 tropical fruits native to Cerrado, Brazil (0.021-1.362 mg of β -carotene/100 g of edible portion) [7], which are considered to be good sources of carotenoids and other bioactive compounds. This difference could be attributed to a number of factors such as, genotype, maturity stage, temperature and light intensity of each geographical region [14].

For the other fruits under study, total carotenoids content was found to be lower than those obtained for mamey sapote: yellow cashew (between 20 and 30 mg of β -carotene/100 g of edible portion); white sapote, red mombin, red cashew and green-yellow mombin (between 10 and 20 mg of β -carotene/100 g of edible portion); green nance, yellow nance and red nance (between 5 and 10 mg of β -carotene/100 g of edible portion); mamoncillo, purple star apple, black sapote, ilama, purple sugar apple, custard apple, sapodilla, green star apple, dragon fruit and green sugar apple (values below 5 mg/100 g of edible portion). The fruits with concentrations below 5 mg of β -carotene/100 g are comparable to the results obtained from the pulp of five tropical fruits grown in Cerrado, Brazil [16].

3.2 Antioxidant Activity of Carotenoids Extract from The Tropical Fruits Evaluated

Table 3 shows the antioxidant activity of carotenoids extract from the edible portion of the group of tropical fruits evaluated, expressed as antioxidant capacity equivalent to Trolox (CAET) (μM Trolox/100 g of edible portion), using ABTS and DPPH assays. Trolox (a water soluble analogue of α -tocopherol) is known for its high antioxidant capacity and is therefore used as a reference compound. Trolox is a compound with greater antioxidant capacity in comparison with ascorbic acid, quercetin, gallic acid and rutin [17]; thus it was used as the standard.

Table 2. Content of total carotenoids of tropical fruits from Yucatan, Mexico.

Fruit	Total carotenoids (mg of β -carotene/ 100 g of edible portion)
Mamey sapote	36.41 \pm 0.01
Yellow cashew	29.37 \pm 0.01
White sapote	19.73 \pm 0.04
Red mombin	17.91 \pm 0.01
Red cashew	17.37 \pm 0.01
Green-yellow mombin	10.32 \pm 0.01
Green nance	9.70 \pm 0.01
Yellow nance	9.05 \pm 0.01
Red nance	8.22 \pm 0.01
Mamoncillo	4.10 \pm 0.01
Purple star Apple	4.05 \pm 0.05
Black sapote	2.56 \pm 0.01
Ilama	1.62 \pm 0.01
Purple sugar apple	1.52 \pm 0.02
Custard Apple	1.20 \pm 0.01
Sapodilla	1.08 \pm 0.06
Green star Apple	1.01 \pm 0.04
Dragon fruit	0.86 \pm 0.01
Green sugar Apple	0.70 \pm 0.03

Values are expressed as mean \pm standard deviation (n=6)

When the ABTS assay was used, the antioxidant capacity of the carotenoids extract found in the fruits evaluated varied from 1.38 to 70.80 μM Trolox/100 g. Among the fruits studied, the red mombin, green sugar apple, green-yellow mombin, yellow cashew, purple sugar apple and green nance (values ranging from 60 to 80 μM Trolox/100 g of edible portion) showed greater antioxidant activity.

For the remainder of the fruits analysed, the antioxidant activity values obtained ranged from 40 to 60 μM Trolox/100 g (green star apple, yellow nance and ilama), from 20 to 40 μM Trolox/100 g (white sapote, red cashew, purple star apple, red nance, custard apple and mamoncillo), and from 0 to 20 μM Trolox/100 g (mamey sapote, black sapote, dragon fruit and sapodilla).

When the DPPH method was used to measure the antioxidant activity of the carotenoids extract from the fruits under study, the edible portion of green nance presented greater antioxidant activity (23.9 μM Trolox/100 g).

Most of the fruits included in this study showed an antioxidant activity of carotenoids extract ranging from 8 to 20 μM Trolox/100 g (sapodilla, purple sugar apple, ilama, green sugar apple, dragon fruit, yellow nance, purple star apple, red cashew, black sapote, green-yellow mombin, green star apple, custard apple and red nance). The antioxidant activity values of these pigments in the rest of the tropical fruits were lower than 8 μM Trolox/100 g (mamoncillo, yellow cashew, red mombin, mamey sapote and white sapote).

Table 3. Antioxidant activity of carotenoid extracts of tropical fruits from Yucatan, Mexico.

Fruit	μM Trolox/100 g of edible portion	
	ABTS ^{•+}	DPPH [•]
Red mombin	70.8 \pm 5.1	3.5 \pm 0.8
Green sugar apple	69.0 \pm 2.5	9.6 \pm 0.5
Green-yellow mombin	66.8 \pm 10.9	14.9 \pm 2.2
Yellow cashew	65.3 \pm 9.7	5.4 \pm 0.1
Purple sugar apple	61.5 \pm 4.7	8.5 \pm 0.4
Green nance	61.1 \pm 4.8	23.9 \pm 2.2
Green star apple	53.0 \pm 2.5	15.6 \pm 0.6
Yellow nance	50.7 \pm 5.0	12.5 \pm 1.1
Ilama	50.6 \pm 5.1	9.1 \pm 0.6
White sapote	38.5 \pm 3.7	1.4 \pm 0.0
Red cashew	35.9 \pm 3.0	13.8 \pm 2.5
Purple star Apple	33.5 \pm 12.2	13.0 \pm 1.1
Red nance	28.3 \pm 3.6	16.4 \pm 1.2
Custard apple	27.3 \pm 0.3	15.8 \pm 3.2
Mamoncillo	27.1 \pm 4.4	5.5 \pm 0.9
Mamey sapote	19.8 \pm 2.8	2.8 \pm 0.0
Black sapote	11.1 \pm 1.4	14.8 \pm 2.6
Dragon fruit	8.9 \pm 0.1	11.4 \pm 0.7
Sapodilla	1.3 \pm 0.0	8.4 \pm 0.7

Values are expressed as mean \pm standard deviation (n=6)

According to these results, the carotenoids extracts of fruits evaluated showed lower antioxidant activity values using the DPPH assay in comparison with the values obtained from the ABTS assay. A possible explication for this could lie in the fact that the DPPH assay was measured at 515 nm (visible region wavelength) and coloured compounds such as the carotenoids present in the fruits show an absorption spectrum similar to that of the DPPH, which can cause interference in the measurement [6]. This result was also reported in a study carried out on the peel, pulp and seeds of the fruit *Canarium odontophyllum* [13].

The antioxidant activity values of carotenoids extract obtained in this work for the 19 fruits evaluated with both assays

(ABTS and DPPH) are similar to or lower than those reported by Kljak and Grbesa [18] and Zanfimi *et al.* [19] for carotenoid extracts from six varieties of corn (*Zea mays L.*), a high yielding hybrid produced in Croatia (values between 58.7 and 89 μM Trolox/100 g of edible portion) and for lipophilic extracts of three varieties of tomato commercialized in a local market of Siena, Italy: Naomi, Ikram and Eroe (values between 9 and 28 μM Trolox/100 g of edible portion), respectively.

It is important to note that the antioxidant activity values of the carotenoids extract from the fruits analysed by both methods, DPPH and ABTS, were found to be lower than the values obtained from the antioxidant activity of soluble phenolic compounds in these same fruits evaluated by Moo-Huchin *et al.* [6]

(values ranging from 359.6 to 684.78 μM Trolox/100 g and from 113 to 380.66 μM Trolox/100 g, for the ABTS and DPPH assays, respectively). However, this result confirms that both bioactive compounds (phenolic and carotenoid compounds) contribute to the antioxidant activity of the fruits grown in Yucatan, Mexico.

3.3 Composition of Individual Carotenoids from The Tropical Fruits Studied

In this research paper, four carotenoids (two xanthophyll's and two carotenes) were identified and quantified by HPLC in the edible portion of a group of tropical fruits grown in Yucatan, Mexico (Table 4). According to the chromatogram of carotenoid standards and fruit samples evaluated, the elution order and retention time of these pigments was: lutein (3.3 min), β -cryptoxanthin (6.3 min), lycopene (7.5 min) and β -carotene (13.5 min) (Figure 1).

Table 4. Carotenoids contents of nineteen tropical fruits from Yucatan, Mexico.

Fruit	Xanthophylls		Carotenes		Vitamin A
	Lutein ($\mu\text{g}/100 \text{ g}^*$)	β -cryptoxanthin ($\mu\text{g}/100 \text{ g}$)	Lycopene ($\mu\text{g}/100 \text{ g}$)	β -carotene ($\mu\text{g}/100 \text{ g}$)	RE ($\mu\text{g}/100 \text{ g}$)
Custard apple	11.0 \pm 0.3	n.d	12.7 \pm 0.0	64.1 \pm 1.8	10.7 \pm 0.3
Ilama	11.7 \pm 0.2	n.d	n.d	80.6 \pm 1.7	13.4 \pm 0.2
Purple star apple	3.9 \pm 0.0	n.d	2.6 \pm 0.1	151.8 \pm 20.7	25.3 \pm 3.4
Green star apple	1.1 \pm 0.0	n.d	3.4 \pm 0.2	93.3 \pm 17.2	15.5 \pm 2.8
Sapodilla	2.9 \pm 0.0	0.2 \pm 0.0	0.5 \pm 0.1	58.8 \pm 8.7	9.8 \pm 1.4
Red mombin	530.2 \pm 8.7	13.7 \pm 0.0	29.6 \pm 0.1	3661.5 \pm 34.7	611.4 \pm 5.7
Green-yellow mombin	390.7 \pm 7.1	19.7 \pm 0.7	n.d	3367.5 \pm 143.6	562.9 \pm 23.9
Mamey sapote	14.3 \pm 0.1	4.1 \pm 0.0	720.3 \pm 4.9	2022.9 \pm 4.2	337.5 \pm 0.7
Yellow cashew	11.7 \pm 2.0	11.5 \pm 0.5	66.5 \pm 0.4	17441.8 \pm 448.3	2907.9 \pm 74.6
Red cashew	22.4 \pm 1.4	6.5 \pm 0.0	n.d	11995.0 \pm 69.1	1999.7 \pm 11.5
Yellow nance	551.6 \pm 50.4	n.d	94.4 \pm 10.6	1402.4 \pm 109.8	233.7 \pm 18.3
Red nance	288.3 \pm 16.3	n.d	8.5 \pm 0.3	1147.6 \pm 114.8	191.2 \pm 19.1
Green nance	406.2 \pm 8.9	n.d	11.0 \pm 2.1	2311.8 \pm 35.0	385.3 \pm 5.8
Dragon fruit	30.8 \pm 0.3	0.6 \pm 0.0	3.2 \pm 0.6	209.1 \pm 0.1	34.9 \pm 0.0
Purple sugar apple	4.3 \pm 0.0	n.d	0.6 \pm 0.0	n.d	0.0 \pm 0.0
Green sugar apple	4.1 \pm 0.0	0.9 \pm 0.0	0.5 \pm 0.0	57.6 \pm 5.4	9.6 \pm 0.9
Mamoncillo	61.6 \pm 1.2	3.5 \pm 0.6	n.d	7598.5 \pm 442.0	1266.7 \pm 73.7
White sapote	10.8 \pm 0.3	0.9 \pm 0.0	0.6 \pm 0.0	352.1 \pm 22.7	58.7 \pm 3.8
Black sapote	34.2 \pm 3.0	9.3 \pm 0.9	2.1 \pm 0.1	290.4 \pm 22.8	49.1 \pm 3.8

Values are expressed as mean \pm standard deviation (n=6)

*Edible portion

n.d: not detected

RE= Retinol equivalent (μg b-carotene/6) + (μg b-cryptoxanthin/12)

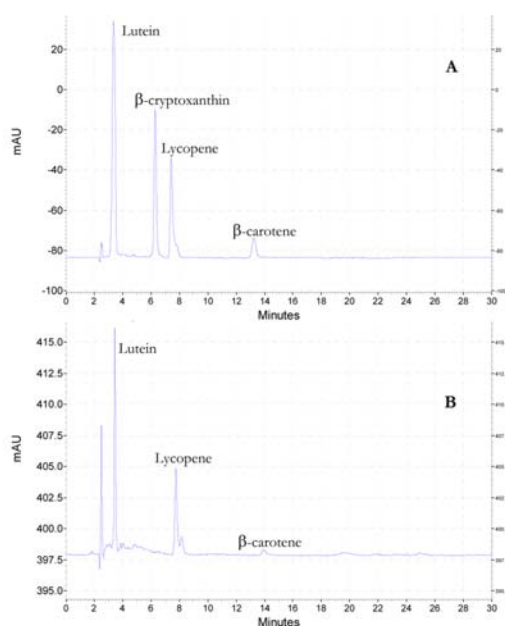


Figure 1. HPLC chromatograms for carotenoids. Carotenoid standards (A); Custard apple (B).

The yellow cashew presented the highest level of β -carotene, with a concentration of 17,441.8 $\mu\text{g}/100$ g of edible portion (Table 4). Lutein, classified as xanthophylls, was detected in all the fruits studied and was also the most abundant pigment found in yellow nance (551.66 $\mu\text{g}/100$ g of edible portion). Scientific research has revealed that low levels of lutein in blood plasma have been associated with a greater risk of cardiac arrest [20]. Other reports indicate that lutein is responsible for maintaining a normal visual function of the macula in the human eye [21]. In the macula, lutein and zeaxanthin absorb blue light and mitigate the effects of the photo-oxidation caused by excessive blue light, thereby reducing chromatic anomalies of the eye. In people over 64 years of age, visual sensitivity depends directly on the concentrations of lutein and zeaxanthin in the retina [22]. Since lutein cannot be synthesized by humans and must be obtained through the ingestion of foods containing

this compound, consumption of the 19 fruits included in this study is recommended given their high content of this pigment.

β -cryptoxanthin, a xanthophyll, was detected in 11 of the 19 fruits (values ranging from 0.25 to 19.74 $\mu\text{g}/100$ g of edible portion), and was the predominant pigment in the green-yellow mombin.

β -carotene, classified as carotenes, was found in 18 of the 19 fruits (values between 57.60 and 17,441.88 $\mu\text{g}/100$ g of edible portion), and was the most abundant compound in the yellow cashew.

Carotenoids such as β -carotene, α -carotene, γ -carotene and β -cryptoxanthin, are precursors of vitamin A, a potent antioxidant, and regulate the pathogenesis of various chronic degenerative diseases. Structurally, the vitamin (retinol) is half of the β -carotene molecule. This carotenoid is the most potent provitamin A and is widely distributed in many foods [23].

Furthermore, among the group of carotenes, lycopene was found in 15 of the 19 fruits analysed. The mamey sapote presented the highest lycopene content (720.35 $\mu\text{g}/100$ g of edible portion), in comparison with all the other fruits studied herein.

Lycopene does not exhibit provitamin A activity and several studies have demonstrated its capacity to eliminate singlet oxygen [24]. A number of studies have shown that lycopene has a protective effect on lipids, low density lipoproteins (LDL), proteins and DNA molecules against the attack of free radicals, playing an essential role in protecting against diseases caused by oxidative stress [25].

The carotenoids composition of the 19 fruits studied is comparable to those reported for 75 fresh fruits consumed in Japan, such as watermelon, guava, papaya cv., fruit tower, grapefruit cv., star ruby grapefruit,

melon, loquat cv., Fusahikari, Mexican mango, etc. [26].

Similarly, the levels of lutein, β -cryptoxanthin, lycopene and β -carotene reported in this paper are comparable to those of fruits and vegetables of Brazil, such as acerola, camu-camu, yellow cashew, red cashew, guava, mango, melon, papaya, watermelon, chili pepper, pumpkin, tomato and lettuce, among others [27]. These results indicate that the fruits studied in this work are a source of the main carotenoids of importance for human health. The information generated in this paper facilitates available data, which could be of use in estimating the intake of carotenoids from fruits grown in Yucatan, Mexico.

It is important to note that the individual carotenoid composition of the tropical fruit reported in this work, such as yellow nance and mamey, differ from those reported by Mariutti *et al.* [28] and Murillo *et al.* [8] for the same fruit. This difference could be attributed to the use of different types of HPLC columns for the separation of pigments and the pore size of the column [29]. Khachik *et al.* [30] report that C18 columns (as used in this study) affect selectivity and show poor resolution in the separation of geometric isomers of carotenoids; whereas C₃₀ polymeric columns favor high resolution in the separation of carotenoid isomers [31].

In this study, no correlation was observed between the content of each carotenoid and the antioxidant activity measured with the DPPH assay; while the contents of β -cryptoxanthin ($R=0.31$, $P\leq 0.05$) and lutein ($R=0.41$, $P\leq 0.05$) showed low correlation with the antioxidant activity measured with the ABTS assay. These results suggest that β -cryptoxanthin and lutein contents can contribute to the antioxidant activity of the carotenoids of the fruits under study. However, it is possible that other

bioactive substances present in the fruit extract or the synergic interaction between individual carotenoids and other antioxidants may be contributing to the antioxidant activity.

Vitamin A deficiency (VAD) is one of the most common and most devastating deficiencies in the world, particularly in developing tropical countries. Recent reports have indicated that around 4 million children suffer from severe VAD, including 250,000-500,000 who experience xerophthalmia and partial or total blindness as a result of VAD [32].

In this work, significant levels of β -cryptoxanthin and β -carotene were found in the fruits analysed and both possess provitamin A activity; therefore, the biological activity of vitamin A for these carotenoids was calculated and expressed in terms of equivalent to μg of retinol (RE)/100 g of edible portion (Table 4). Vitamin A activity was calculated in terms of retinol equivalents based on the *in vivo* conversion factor proposed by the OMS and NRC [33, 34], in which 1 RE = 1 μg of retinol = 6 μg of β -carotene or 12 μg of β -cryptoxanthin.

Provitamin A activity of the carotenoids from the fruits under study was found in a range of 0-2,907.94 RE in 100 g of edible portion. Of all the fruits studied, the purple sugar apple was found to have no carotenoids with provitamin A activity. The mamoncillo (1,266.72 $\mu\text{g}/100$ g of edible portion), red cashew (1,999.72 $\mu\text{g}/100$ g of edible portion) and yellow cashew (2,907 $\mu\text{g}/100$ g of edible portion) obtained high RE values in comparison with the other fruits.

Red nance, yellow nance, mamey sapote, green nance, green-yellow mombin and red mombin showed moderate RE values (ranging from 191.27 to 611.41 $\mu\text{g}/100$ g of edible portion). All of these results are higher than those found for different cultivars of banana in India (values from 1.91 to 106.0 μg

RE/100 g of edible portion) [35].

Green sugar apple, sapodilla, custard apple, ilama, green star apple, purple star apple, dragon fruit, black sapote and white sapote exhibited values below 60 µg RE/100 g of edible portion. This last group of fruits showed higher RE values in comparison with those reported for 94 cultivars of fruits of the species *Artocarpus* [36].

These results suggest that the group of fruits studied herein show considerable levels of retinol equivalents that could represent a significant contribution to vitamin A requirements in the diet of the local population.

4. CONCLUSIONS

In this study, significant levels of carotenoids with antioxidant activity were found in 19 tropical fruits from Yucatan, Mexico. These pigments not only contribute to the attractive colour of the fruits but also are also important due to the provitamin A activity of some of the compounds.

Lutein, β-cryptoxanthin, β-carotene and lycopene were the most abundant pigments in yellow nance, green-yellow mombin, yellow cashew and mamey sapote, respectively.

There was a positive correlation between antioxidant activity and β-cryptoxanthin and lutein by the ABTS.

Generally speaking, it is possible to conclude that these results indicate a perspective for the exploitation and use of these tropical fruits in the diet, given their antioxidant activity and the content of specific carotenoids most beneficial in conserving human health.

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