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Phytochemical and antioxidant characterization of mamey (*Pouteria sapota* Jacq. H.E. Moore & Stearn) fruit

Elhadi M. Yahia *, Fabiola Gutiérrez-Orozco, Claudia Arvizu-de Leon

Phytochemistry and Nutrition Laboratory, Facultad de Ciencias Naturales, Universidad Autonoma de Queretaro, Avenida de las Ciencias S/N, Juriquilla, 76230, Querétaro, Qro., Mexico

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ABSTRACT

Phytochemical compounds in fruits and vegetables have gained great importance in the last few years because of the increasing evidence suggesting their antioxidant and prevention of chronic diseases. Carotenoids, phenolics, flavonoids, and vitamins E and C, are among these phytochemicals. Several fruits have been characterized so far for their antioxidant and health properties but there is still limited information on fruits from the tropic. Therefore, the objective of this study was the characterization of mamey fruit (*Pouteria sapota* Jacq. H. E. Moore & Stearn) with regard to their antioxidant capacity and phytochemical profile. Phenolics, carotenoids and δ-tocopherol were quantified and identified by HPLC–DAD–Mass Spectrometry (LC–MS), and DPPH and FRAP assays were used to evaluate antioxidant capacity. Hydrophilic extracts of mamey fruit showed higher antioxidant capacity than the lipophilic portion. Total soluble phenols content was 28.5 mg GAE/100 g fw, being *p*-hydroxybenzoic acid as the main phenolic that was identified. Total carotenoid content was 1127.9 μg β-carotene/100 g fw with β-carotene being the main contributor, in addition to lutein, and violoxanthin. Concentration of δ-tocopherol was 360.0 μg/100 g fw. Results of this study suggest that mamey fruit is a good source of carotenoids and its inclusion in the diet is recommended.

1. Introduction

The incidence of chronic degenerative diseases such as cancer, hypertension, cardiovascular disease, obesity, and diabetes has increased in the last decades as a result of changes of diet and lifestyle habits, among other possible causes. Growing scientific evidence suggests an inverse relationship between the consumption of fruits and vegetables and the development of these disorders (Yahia and Ornelas-Paz, 2010; Ames, Shigenaga, & Hagen, 1993; Arts & Hollman, 2005; Dillard & German, 2000; Eastwood, 1999; Esterbauer, Dieber-Rotheneder, Striegl, & Waeg, 1991; Ness & Powles, 1997; Prior & Cao, 2000; Riboli & Norat, 2003; Verlangieri, Kapeghian, el-Dean, & Bush, 1985). In addition to providing essential nutrients, fruits and vegetables also contain several other phytochemicals which have been suggested as responsible for health benefits due to their antioxidant properties and other positive effects (Yahia and Ornelas-Paz, 2010; Wu, Beecher, et al., 2004; Wu, Gu, et al., 2004; Di Majo et al., 2005; da Costa, Ballus, Teixeira-Filho, & Teixeira Godoy, 2010). Phytochemicals are capable of neutralizing the effects of free radicals, thought to be associated with damage to proteins, DNA, cell membranes, etc. giving raise to chronic diseases associated with aging (Ames et al., 1993). Among these compounds, phenolics are believed to provide, at least in part, this antioxidant capacity (AOC) (Duthie, Duthie, & Kyle, 2000; Wang, Cao, & Prior, 1996). Other phytochemical compounds include carotenoids, which have also been related to prevention of several chronic diseases (Krinsky, Landrum, & Bone, 2003; Stahl & Sies, 2005; Yahia, 2010; Yahia and Ornelas-Paz, 2010). In addition, vitamins C and E contribute to the antioxidant capacity of foods (Galano, Vargas, & Martínez, 2010; Pellegrini et al., 2007).

Although several fruits have been studied, characterization of fruits from the tropic is still very limited (Sreeramulu & Raghunath, 2010). Mamey (*Pouteria sapota* H.E. Moore & Stearn) is a tropical fruit from the Sapotaceae family, native to Mexico and Central America. The fruit of mamey has received little attention and research in spite of its popularity in production areas, high potential for commercialization in international markets, and the high carotenoid content found in the pulp (Morton, 1987) making it nutritionally attractive, especially in areas where vitamin A deficiency is a problem. Thus, the characterization of phytochemicals especially those with antioxidant activity, such as phenolics, carotenoids, and vitamins E and C in mamey fruit is very important. The objective of this work was the characterization of fruits of mamey with regard to their antioxidant capacity and phytochemical content by using high performance liquid chromatography–mass spectrometry.

2. Materials and methods

2.1. Reagents

Reagents were obtained from Sigma-Aldrich (St. Louis, Mo., U.S.A.) unless otherwise stated. Standard purity was ≥97% for β-carotene,

* Corresponding author. Phytochemistry and Nutrition Laboratory, Faculty of Natural Sciences, Autonomous University of Queretaro, Avenida de las Ciencias, Juriquilla, 76230, Querétaro, Qro., Mexico. Tel.: +52 442 192 1200x5304; fax: +52 442 234 2958.
E-mail address: yahia@uaq.mx (E.M. Yahia).

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≥79% for lutein, ≥90% lycopene, ≥95 and ≥90% for α- and δ-tocopherol, respectively. Phenolic acid standards were HPLC (high-performance liquid chromatography) grade. The following individual phenolic acids standards were purchased from Sigma Aldrich: cinnamic, gallic, protocatechuic, catechin, chlorogenic, sinapic, quercetin, *p*-hydroxybenzoic, *p*-coumaric, caffeic, kaempferol, ferulic, myricetin, vanillic, epicatechin, isoramnethin, and *o*-coumaric. HPLC-grade methanol, acetone, *n*-hexane, toluene, methyl tert-butyl ether (MTBE), methylene chloride, acetonitrile, and ethanol were purchased from J.T.Baker (Baker Mallinckrodt, Mexico). All other solvents were ACS grade. HPLC-grade water was prepared by a Milli-Q Plus purification system (Millipore Corp., Bedford, MA, USA).

2.2. Samples

Fresh mamey fruits were obtained from the local market in Queretaro, Mexico and were selected on the basis of ripeness, freedom from defects, and uniformity of size and color. The fruits were taken to the Laboratory of Phytochemicals and Nutrition of the Faculty of Natural Sciences of the Autonomous University of Queretaro, where they were washed and physically and chemically characterized by measuring weight, internal and external colors, and total soluble solids content (TSS, °Brix), and representative samples were frozen in liquid nitrogen and freeze-dried until reaching a constant weight. °Brix was measured in the juice obtained from a representative portion of each fruit, using a temperature adjusted hand refractometer (ATAGO, Co. Ltd., Osaka, Japan). Flesh (internal) color was measured with a CM-2002 Minolta spectrophotometer operating with the spectraQC 7.2 software (Minolta, Co. Ltd., Osaka, Japan), which was calibrated with the white pattern during each sampling time. A big slice from a flat side of each fruit (without seed) was obtained and color was determined longitudinally on three equidistant points. L, a*, b*, chroma (C), and hue (h°) values were recorded. Firmness was measured on six points of each fruits after eliminating the skin, using a FT 327 (QA Supplies, Italy) fruit pressure tester with an 8 mm tip. Samples were then frozen with liquid nitrogen and were kept at −80 °C until lyophilization before analysis.

2.3. Extracts preparation and antioxidant capacity analyses

Lipophilic (LPE) and hydrophilic extracts (HPE) were obtained as reported (Wu, Beecher, et al., 2004; Wu, Gu, et al., 2004), with some modifications. Samples of 1 g of freeze-dried powder were homogenized in 10 mL of hexane/dichloromethane (1:1, v/v) using an Ultra Turrax model T25 Basic homogenizer (IKA Works, Willmington, NC, USA). The homogenate was sonicated for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT, USA) and then centrifuged at 15,000×*g* for 10 min at 4 °C. The supernatant was collected, and the sediment was subjected to an additional extraction using the same procedure. Both supernatants were mixed and rotoevaporated at 40 °C. The dried extract was resuspended in 10 mL of HPLC-grade acetone, filtered through a 0.45 μm nylon membrane, and designated as LPE. The residue, after the second extraction process, was homogenized in 20 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v), sonicated, and centrifuged using the same conditions mentioned above. The supernatant was collected, and the sediment was subjected to extraction again. Both supernatants were mixed and designated as hydrophilic extract (HPE). LPE and HPE were used for antioxidant capacity (AOC) analysis.

DPPH (2, 2′-diphenyl-1-picrylhydrazyl) assay was performed as previously reported (Kim, Lee, Lee, & Lee, 2002), with some modifications, using a microplate reader. Aliquots of 280 μL of 100 μM DPPH/methanol solution per well were placed in a 96-well plate, and 20 μL of extracts, diluted to different concentrations, were added to each well to complete 300 μL. Aliquots of 300 μL of methanol were used as a blank. The plates were incubated for 30 min in the dark, and readings were taken at 490 nm in a MRX microplate reader (Dynex Technology, Chantilly, VA, USA) Results (AOC) were expressed as ascorbic acid equivalents (AAE) in mg/100 g fresh weight (fw) (Rice-Evans & Miller, 1994).

For FRAP (ferric ion reducing antioxidant power) assay (Benzie & Strain, 1996), aliquots of 280 μL of FRAP reagent were placed in 96-well plates, and 20 μL of extracts, diluted to different concentrations, were added. The plates were incubated for 30 min in the dark and read at 630 nm in a MRX microplate reader (Dynex Technology). Calibration curves were prepared using ascorbic acid as a standard, and results were expressed as AAE in mg/100 g fw. FRAP reagent was prepared by mixing 50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 10 mM 2, 4, 6-tripyridyl-2-triazine (TPTZ) in 40 mM HCl, and 5 mL of 20 mM $FeCl_3$.

2.4. Analysis of antioxidants compounds

Vitamin E was determined as described previously (Ornelas-Paz, Yahia, & Gardea-Bejar, 2007). Samples of 0.5 g of freeze-dried powder were homogenized in 10 mL of HPLC grade methanol, stirred at 55 rpm in a water bath at 30 °C for 15 min, and centrifuged at 5000×*g* for 5 min. The supernatant was filtered through a 0.45 μm nylon membrane, and 20 μL were injected into the HPLC system (HP 1100 Series, Hewlett-Packard/Agilent Technologies Co., Palo Alto, CA, USA). A 150×4.6 mm i.d., 3.5 μm, Symmetry C18 column (Waters Co., Milford, CT, USA) was used. HPLC-grade methanol (100%) was employed as the mobile phase at a flow rate of 0.8 mL/min. For α and γ-tocopherols detection, a model FLD G1321A fluorescence detector (Agilent Technologies Co., Palo Alto, CA, USA) at an excitation wavelength of 294 nm and emission wavelength of 325 nm was used. Calibration curves for quantification were prepared using standards of α- and γ-tocopherols, respectively. The concentration range and correlation coefficients (r^2) for the calibration curves were 0–0.1 mg/mL and 0.9989, and 0–0.1 mg/mL and 0.9987 for α- and γ-tocopherols, respectively.

Total soluble phenols (TSP) were extracted as described before (Wolfe, Xianzhong, & Liu, 2003), with some modifications. Samples of 1 g of freeze-dried powder tissue were homogenized in 20 mL of 80% acetone using an Ultra Turrax model T25 basic homogenizer (IKA Works) at room temperature. The homogenate was subjected to sonication for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co.) and centrifugation at 19,000×*g* for 15 min at 2 °C in a Hermle Z323K centrifuge (Labortechnik, Wehingen, Germany). The supernatant was collected, and an additional extraction was done in the sediment following the same procedure. Both supernatants were mixed and evaporated at 40 °C using a rotary evaporator (Buchi R-205, Labortechnik, Switzerland). The concentrate was diluted with 25 mL of methanol and taken to 50 mL with HPLC-grade water. This was filtered through a 0.45 μm membrane, and aliquots were taken for analysis. The extraction process was performed in triplicate.

For TSP quantification, aliquots were diluted with HPLC-grade water (1:10), and 30 μL of sample per hole was placed in 96-well plates, and 150 μL of Folin-Ciocalteu reagent (dilution 1:10) and 120 μL of 7.5% Na_2CO_3 were added. The plates were incubated for 2 h in the dark, and absorbance at 630 nm was measured using a Dynex MRX microplate reader (Dynex Technol.). Results were expressed as milligrams of gallic acid equivalents (GAE)/100 g fw.

Identification and quantification of individual phenolics were carried out using HPLC analysis. The phenols extracts were prepared as mentioned before. Aliquots of 20 μL were injected into the HPLC system (HP 1100 Series, Hewlett-Packard/Agilent Technologies Co.) equipped with a diode array detector (DAD) set at 280 and 320 nm. A 250×4.6 mm i.d., 5 μm, X-terra RP18 column (Waters) was used. Mobile phase consisted of 1% formic acid (98%) (A) and acetonitrile (2%) (B), flow rate of 0.5 mL/min. Elution gradient was 2 to 100% (B) from 0 to 70 min. Calibration curves for each standard were prepared

for quantification. Characterization of the phenolic extracts was carried out by coupling the HPLC system to an HP 6210 time of flight mass spectrometer (MS–TOF, Agilent Technologies Co.), equipped with an electrospray ionization interface (ESI) operating at the negative ionization mode with the following settings: drying gas temperature (nitrogen): 350 °C, drying gas flow rate: 9 L/min, nebulizer pressure: 40 psig, fragmentor voltage: 220 V, skimmer: 60 V, capillary voltage: 3500 V, and scan range of *m/z*: 50–1000. For injection of mamey samples into the LC–ESI–MS system, extracts were previously hydrolyzed by mixing 2 mL of phenolic extract with 2 mL of 2.4 M HCl. The mixture was then heated at 80 °C for 2 h. After that, it was cooled down and filtered before injection.

Total carotenoid (TC) extraction was performed using the 970.64-AOAC method (AOAC, 2000), with some modifications (Soto-Zamora, Yahia, Brecht, & Gardea, 2005). Briefly, samples of 0.5 g of the freeze-dried sample were mixed with 10 mL of hexane/acetone/toluene/ethanol (10:7:7:6, v/v/v/v) solution and 1 mL of 40% KOH in methanol, stirred at 56 °C for 20 min, and cooled with tap water and then 10 mL of hexane were added. After that, 10 mL of 10% Na_2SO_4 was added, stirred, and the mixture was incubated in the dark until phase separation. The top phase was taken for analysis. TC quantification was measured in a Beckman DU-65 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, U.S.A.) set at 450 nm. A calibration curve was established using β-carotene in hexane as the standard and hexane as the blank.

For β-carotene and lutein quantifications, aliquots were filtered through a 0.45 μm nylon membrane (Pall Co., Ann Arbor, MI, USA) and injected (20 μL) into the HPLC, equipped with a DAD set at 470 nm. A 150 × 4.6 mm i.d., YMC C30 column (YMC Inc., Wilmington, NC, USA) was used. The mobile phase consisted of methanol (100%) and methyl-terbutyl-ether (MTBE, 0%). The elution gradient was 0–100% MTBE in 35 min at a flow rate of 1 mL/min. For quantification, a calibration curve was prepared using β-carotene and lutein. Measurements were performed in triplicate. Characterization of the carotenoid extracts was carried out by coupling the HPLC system to an HP 6210 time of flight mass spectrometer (LC–MS–TOF.). An atmospheric pressure chemical ionization ($ApCI^+$) source operating at the positive ionization mode was used. Ionization parameters were as follows: drying gas temperature (nitrogen): 350 °C, drying gas flow rate: 5 L/min, corona voltage: 4.0 μA, nebulizer pressure: 20 psig, fragmentor voltage: 200 V, skimmer: 60 V, capillary voltage: 4000 V, and scan range of *m/z*: 100–1000.

2.5. Data analyses

Results were presented as means of at least three replications and the standard deviation from the mean. All data analyses were performed using Sigma Plot 10.0 (Systat Software Inc, San Jose, CA, USA).

Table 1
Total soluble solids content (°Brix), weight, firmness, and internal (pulp) color in mamey fruit.[a]

Parameter	Value
Total soluble solids (°Brix)	9.8 ± 3.1
Weight (g)	544.26 ± 90.89
Firmness (kg)	5.5 ± 2.5
Color	
L	65.862 ± 6.508
a*	24.418 ± 5.439
b*	30.312 ± 4.03
Hue (h)	51.379 ± 7.556
Chroma (C)	39.222 ± 4.638

[a] Data are means of three repetitions ± standard deviation (SD).

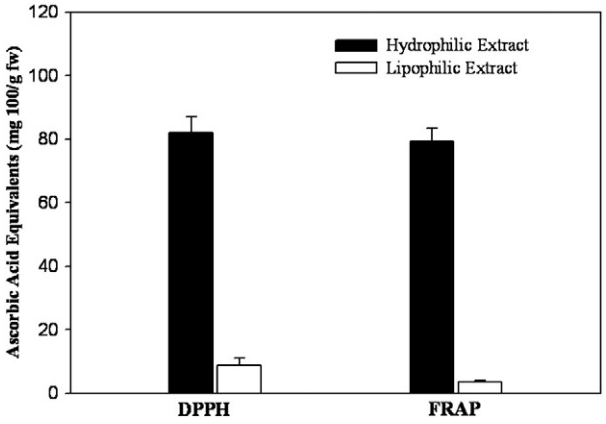

Fig. 1. Antioxidant capacity of hydrophilic and lipophilic extracts from mamey fruit measured by DPPH and FRAP assays.

3. Results and discussion

Physical characteristics of mamey fruit used in this study such as color, weight, firmness and total soluble solids (°Brix) are presented in Table 1. Total soluble solids content (TSS) in mamey fruit (9.8 °Brix), and color parameters (L, h° and C) were in the same range as those reported previously; pulp color being reddish orange (Gomez-Jaimes et al., 2009).

AOC of mamey fruit that was measured using DPPH and FRAP assays gave similar results (Fig. 1). HPE had higher AOC than LPE using both assays (HPE: 82.016 ± 4.99, LPE: 8.700 ± 2.38 mg AAE/100 g fw by DPPH; and HPE: 79.290 ± 4.15, LPE: 3.465 ± 0.407 mg AAE/100 g fw by FRAP). When AOC was measured in different crops (guava, avocado, black sapote, mango, papaya, prickly pear fruit, cladodes, and strawberry), HPE were found to possess higher AOC than LPE as measured by different assays (DPPH, FRAP, TEAC (trolox equivalent antioxidant capacity), ORAC (oxygen radical absorbance capacity), TOSC (total oxidant scavenging capacity), and DMPD (*N,N*-dimethyl-*p*-phenylendiamine) (Corral-Aguayo, Yahia, Carrillo-Lopez, & Gonzalez-Aguilar, 2008). It is recommended to use more than one assay to assess AOC in order to get better accuracy of the results (Dini, Tenore, & Dini, 2009). Here, we found that AOC values by DPPH were similar to those given by FRAP. The former is widely accepted because of stability, reproducibility and simplicity (Katsube et al., 2004). The fact that LPE presented low AOC as compared to the HPE could be due to the limited ability of DPPH and FRAP to detect other compounds with antioxidant activity such as carotenoids, which is important in mamey (Benzie & Strain, 1999). Thus, another assay for measuring AOC in LPE of mamey may be necessary. Comparison of results among different reports is often difficult since many assays are employed to evaluate the antioxidant capacity of fruits and vegetables and they generally use different substrates and reaction kinetics (Benzie & Strain, 1999; Cao & Prior, 1999; Gil, Tomas-Barberan, Hess-Pierce, Holeroft, & Kader, 2000; Evelson, Travacio, & Repetto, 2001; Van den Berg, Haenen, Van den Berg, & Bast, 1999). An important factor

Table 2
Contents of antioxidant compounds in mamey fruit.[a]

Compound	Content
TSP (mg GAE/100 g fw)	28.507 ± 0.614
TC (μg β-carotene/100 g fw)	1127.936 ± 5.298
δ-Tocopherol (mg/100 g dw)	0.360 ± 0.030

TSP: Total soluble phenols.
TC: Total carotenoids.
fw: Fresh weight.
dw: dry weight.
[a] Data are means of three repetitions ± standard deviation (SD).

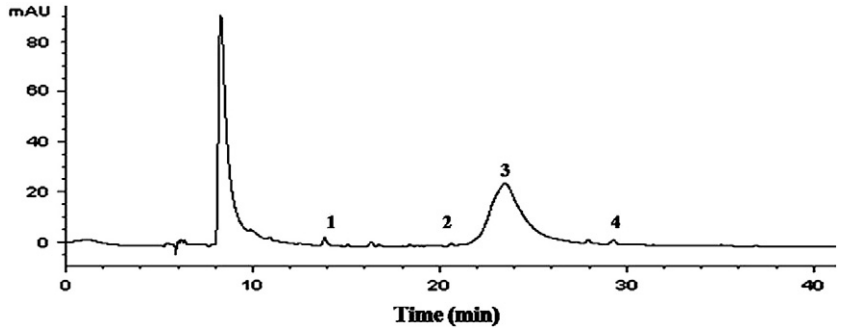

Fig. 2. HPLC chromatogram at 280 nm of mamey fruit phenolics extract: 1: *p*-hydroxybenzoic acid; 2: gallic acid; 3: epicatechin; and 4: protocatechuic acid.

to consider when interpreting AOC results is that differences may exist between the observed *in vitro* AOC and the actual *in vivo* effects.

No α-tocopherol was detected in mamey, while the content of δ-tocopherol was lower than what has been reported for other fruits such as guava, mango and strawberry (Corral-Aguayo et al., 2008) (Table 2). According to the recommended dietary intake for vitamin E for the Mexican population (Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, 2001), the contribution of mamey is very low. However, a synergistic antioxidant effect of δ-tocopherol with flavonoids such as (−)-epicatechin and (−)-epigallocatechin gallate (EGCG) has been reported (Kadoma, Ishihara, Okada, & Fujisawa, 2006), and therefore even low concentrations of δ-tocopherol in mamey may be important considering the concomitant presence of epicatechin in mamey as shown below.

TSP content (28.5068 ± 0.614 mg GAE/100 g fw) was lower than what has been reported for other fruits like apples, oranges, strawberries, and others, but higher than the TSP content found in honeydew melon (Chun, Kim, Smith, Schroeder, & Han, 2005) (Table 2). However, when compared to other *Pouteria* species (*Pouteria campechiana* and *Pouteria viridis*), *Pouteria sapota* presented the highest phenolic content (Ma, Yang, Basile, & Kennelly, 2004). Mustafa, Abdul Hamid, Mohamed, and Abu Bakar (2010) reported higher TSP contents in 21 tropical fruits, ranging from 45.96 to 728.43 mg GAE/g, than what we have found here for mamey fruit.

HPLC analyses revealed that the main phenolics in mamey fruit corresponded to three hydroxybenzoic acids (*p*-hydroxybenzoic, protocatechuic and gallic), and a flavan-3-ol (epicatechin) (Fig. 2). The phenolic *p*-hydroxybenzoic acid was found in the highest concentration (484 mg/100 g dw). There was 1.92 mg/100 g dw gallic acid, 0.78 mg/100 g dw epicatechin, and 2.08 mg/100 g dw protocatechin. Previous studies have reported the following phenolics in mamey: gallic acid, gallocatechin, catechin, epicatechin, dihydromyricetin, catechin-3-*O*-gallate, and myricetin. From these, dihydromyricetin was reported in the highest concentration (Ma et al., 2004). With the exception of red fruits such as black radish and onions, the content of *p*-hydroxybenzoic acid in edible plants is usually very low. In strawberry, for instance, *p*-hydroxybenzoic acid concentration of 20–90 mg/kg fw has been reported (Shahidi & Naczk, 1995). Although in much less amount, gallic acid was also detected in mamey. Gallic acid has been associated with chemoprevention, antioxidant and antimicrobial activities (Morais et al., 2010). Tea is one of the best sources of gallic acid (100–800 mg/L) (Hertog, Hollman, & van de Putte, 1993). Together with catechin, epicatechin is the main flavan-3-ol in fruits (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). Levels of epicatechin in red grape, apricot and apples (8.7, 5.5, and 5.5 mg/100 g fw, respectively) have been previously reported (US Department of Agriculture, 2007). Regarding protocatechuic acid, levels found here are higher than what has been previously reported in mandarin peels (Ma et al., 2008) and blueberries (Ayaz, Hayirlioglu-Ayaz, Gruz, Novak & Strnad, 2005).

Confirmation and further identification of phenolic compounds in mamey fruit was carried out in hydrolyzed extracts by LC–ESI–MS. Total ion chromatogram (TIC) is shown in Fig. 3 and peak identification is given in Table 3. Peak 1 was identified as gallic acid according to retention time (T_r) of 11.7 min and fragmentation pattern presenting the *m/z* at 124 as base ion with another fragment at *m/z* 168. Peak 2 with fragments at *m/z* 197, 182 and 123 was identified as syringic acid according to previous reports (Fang, Yu, & Prior, 2002). Peak 3 was identified as *p*-hydroxybenzoic acid given by the ions at *m/z* 138, 137 and 120 (loss of water molecule). *p*-Hydroxybenzoic acid was also

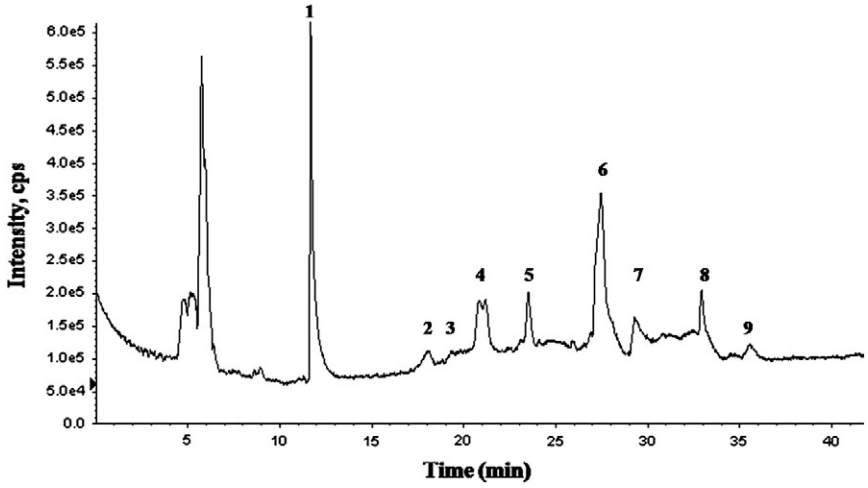

Fig. 3. Total Ion Chromatogram (TIC) of hydrolyzed phenolic extracts from mamey fruit. For peak identification refer to Table 3.

Table 3
Phenolic compounds identified in mamey fruit by LC–ESI–MS analyses.[a]

Peak	T_r (min)	*m/z*	Compound name
1	11.7	124, 168	Gallic acid
2	18.0	197, 182, 123	Syringic acid
3	19.2	138, 137, 120	*p*-Hydroxybenzoic acid
4	20.8	580	Epicatechin dimer
5	23.5	196, 164, 136	Unknown
6	27.4	164, 120	*p*-Coumaric acid
7	29.3	357, 154, 136	Protocatechuic hexose-malonate
8	32.8	137, 155, 353	Hydroxybenzoic acid derivative
9	35.6	137, 155, 258	*p*-Hydroxybenzoic acid dimer

[a] Peak numbers and retention times correspond to Fig. 3.

identified in the non-hydrolyzed extracts with fragments at *m/z* 138 and 385 which suggests *p*-hydroxybenzoic is naturally found to be linked to hexose-malonate (data not shown). Peak 4 ($T_r = 20.8$ min) had a molecular ion at *m/z* 580 and was tentatively identified as an epicatechin dimer. Fragments at *m/z* 164 and 120 were identified in peak 6 ($T_r = 27.4$ min), corresponding to *p*-coumaric acid, which could have been masked by *p*-hydroxybenzoic acid during the HPLC run. Peak 7 was identified as protocatechuic hexose-malonate with *m/z* fragments at 357 (loss of carboxyl group), 154 (free mass of the acid), and 136 (loss of water molecule). Peak 8 was tentatively identified as derivative of hydroxybenzoic acid given by the ion fragments at *m/z* 137, 155, and 353 (Rubilar, Pinelo, Shene, Sineiro, & Nuñez, 2007). Peak 9 with fragments at *m/z* 137, 155, and 258 could be a dimer of *p*-hydroxybenzoic acid.

The effects of phenolics on different chronic disease have been subject of study in the last few years, especially with regard to their antioxidant properties. For example, *p*-hydroxybenzoic acid has been shown to have potent anti-inflammatory *in vitro* activity (Choudhary et al., 2009). Epicatechin has been suggested as responsible for the increase in plasma antioxidant capacity and quercetin as a preventing agent against LDL oxidation and lung cancer (Lamuela-Raventós, Andrés-Lacueva, Permanyer, & Izquiero-Pulido, 2001; Knekt et al., 1997). These effects are thought to be exerted through several mechanisms such as free-radical scavenging activity, transition-metal-quelating activity, and/or singlet-oxygen-quenching capacity (Rice-Evans, Miller, & Paganga, 1997).

As shown in Table 2, TC content found in this study was lower than previously reported values (62.53 μg/g, De Rosso & Mercadante, 2007). This difference might be due to different factors such as the extraction method, stage of ripeness, and growing conditions (Corral-Aguayo et al., 2008).

Two carotenoids, β-carotene and lutein, were identified by HPLC analysis by comparison with retention times of the standards (Fig. 4). The fruit had 3.764 mg/100 g β-carotene and 0.087 mg/100 g lutein on a dry weight basis. MS analyses (Fig. 5) of carotenoid extracts resulted in the identification of the following compounds: Peak 1 ($T_r = 7.6$ min) presented ion fragments at *m/z* 671, 653, and 572 and similar retention time as the standard compound, which suggests it may correspond to lutein epoxide. Peak 2 presented similar fragmentation pattern compared to peak 1, with ion fragments at *m/z* 671 and 572 and retention times close to that of lutein, which may suggest it could be an isomer of this compound. Peaks 3, 4, and 5

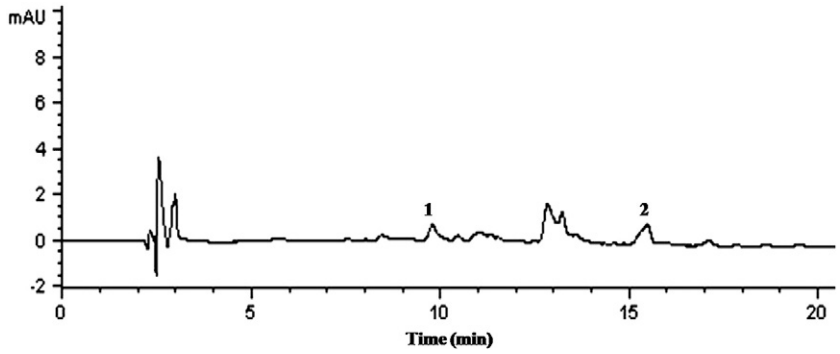

Fig. 4. HPLC chromatogram at 470 nm of mamey fruit carotenoids extracts: 1: lutein; 2: β-carotene.

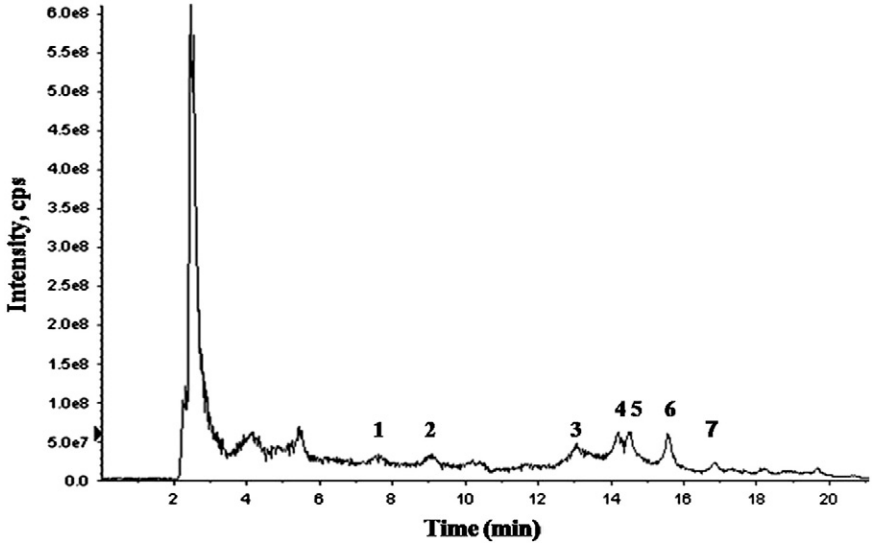

Fig. 5. Total Ion Chromatogram (TIC) of carotenoid extracts from mamey fruit. For peak identification refer to Table 4.

Table 4
Carotenoid compounds identified in mamey fruit by LC–ESI–MS analyses.[a]

Peak	T_r (min)	*m/z*	Compound name
1	7.6	671, 653, 572	Lutein epoxide
2	9.1	671, 572	Isomer of lutein
3	13.0	671, 576, 529, 423, 409	Isomer of β-carotene epoxide
4	14.2	671, 576, 529, 423, 409	Isomer of β-carotene epoxide
5	14.5	671, 576, 529, 423, 409	Isomer of β-carotene epoxide
6	15.5	671, 423	Violoxanthin
7	16.9	961, 669, 530	Lutein-myristate-laurate

[a] Peak numbers and retention times correspond to Fig. 5.

presented ion fragments at *m/z* 669, 576, 529, 423, and 409, which may correspond to epoxides of β-carotene isomers, according to the loss of toluene (*m/z*, 576), epoxide in a β-ring with hydroxyl group (*m/z*, 423), and the fragments at *m/z* 409 and 529, which have been previously reported for β-carotene isomers (Ornelas-Paz et al., 2007). Ion fragments at *m/z* 671 and 423 were identified in peak 6 ($T_r = 15.5$ min) and may correspond to violoxanthin according to previous reports in mango (Ornelas-Paz et al., 2007). Peak 7 at 16.9 min had ion fragments at *m/z* 961, 669, and 533, and was identified as lutein-myristate-laurate according to what has been previously reported (Young, Abdel-Aal, Rabalski, & Blackwell, 2007) (Table 4). Previous studies in mamey from the Amazon found that among carotenoid compounds in the fruit, *all-trans*-β-carotene was in the highest concentration, in addition to *cis*-violoxanthin and luteoxanthin which were found in lesser amounts (De Rosso & Mercadante, 2007).

Lutein is the main carotenoid found in leaves, green vegetables and flowers (Yahia, 2010; Yahia and Ornelas-Paz, 2010). Green vegetables like spinach and broccoli have concentrations of lutein well above the 2000 μg/100 g fw. In mango, guava, and papaya fruits, for instance, lutein concentrations of 100, 270, and 20–282 μg/100 g fw, respectively, have been reported (Setiawan, Sulaeman, Giraud, & Driskell, 2001). Our study revealed concentrations of lutein below the levels found in some tropical fruits. A wide range of β-carotene contents in mango has been observed in different studies. For example, in mangos from Thailand, 60 μg/100 g was reported, while 2900 and more than 6000 μg/100 g were found in India and Brazil (Mercadante & Rodriguez-Amaya, 1998). Here, we observed that the concentration of β-carotene found in mamey is above those found in melon, papaya, and peach fruits (Setiawan et al., 2001). As compared to fruits from temperate climates, it has been suggested that tropical fruits present higher carotenoid content (Yahia, 2010; Yahia and Ornelas-Paz, 2010) and thus, their use and inclusion in the diet is important. Two important carotenoids found in mamey fruit (β-carotene and β-cryptoxanthin) are provitamin A, and therefore mamey may represent an important source of vitamin A in the diet especially in areas where deficiency is likely to be present. In addition, the role of carotenoids in scavenging harmful reactive oxygen species has been suggested (Burton & Ingold, 1984; Van den Berg et al., 2000). This activity may be mediated by quenching singlet oxygen by electron transfer (Young & Lowe, 2001).

4. Conclusions

Phytochemical characterization of mamey fruit in this study revealed the presence of phenolic and carotenoid compounds which may have an important role in maintaining good health according to the antioxidant activity that these compounds confer to the fruit. Their inclusion in the diet as a source of vitamin A is also suggested. Further studies on the biological activities of phytochemicals in mamey are warranted.

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