Identification and quantification of phenols, carotenoids, and vitamin C from papaya (Carica papaya L., cv. Maradol) fruit determined by HPLC-DAD-MS/MS-ESI

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A B S T R A C T

Recent studies have demonstrated that vitamin C, phenols, and carotenoids are bioactive compounds that protect the body from oxidative stress, reducing the risk of cardiovascular diseases and some types of cancer. Qualitative and quantitative analysis of the major phytochemicals found in papaya fruit flesh and skin (Carica papaya L., cv Maradol) was conducted in four stages of ripeness, using high-performance liquid chromatography mass spectrometry. Phenolic compounds identified in the fruit skin tended to decrease with ripening. The compounds identified were ferulic acid (277.49 to 186.63 mg/100 gDW), p-coumaric acid (229.59 to 135.64 mg/100 gDW), and caffeic acid (175.51 to 112.89 mg/100 gDW). The following carotenoids, along with vitamin C, increased in flesh with ripening: lycopene (0.28 to 1.06 mg/100 gDW), β-cryptoxanthin (0.28 to 1.06 mg/100 gDW), β-carotene (0.23 to 0.50 mg/100 gDW), and vitamin C (25.07 to 58.59 mg/100 gDW). These results indicate that stage of ripeness significantly influences the contents of bioactive compounds in papaya fruit.

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1. Introduction

In recent years, consumption of fruits and vegetables has increased considerably because of their benefits for good health (Yahia, 2010). Many biochemical and epidemiological studies have demonstrated that fruits and vegetables contribute to the reduction of several diseases, including cardiovascular, neurological, and carcinogenic illnesses (Stanner, Hughes, Kelly, & Buttriss, 2004). These benefits have been attributed, at least in part, to the amount of antioxidant compounds present in these foods, which reduce the oxidative stress produced by free radicals, and in consequence, cellular damage (Dosil-Diaz, Ruano-Ravina, Gestal-Otero, & Barros-Dios, 2008). Some of the most important antioxidant compounds present in fruits and vegetables include polyphenols, carotenoids, and vitamin C (Yahia, 2010).

Phenolic compounds are aromatic metabolites of plants secondary metabolism that have a common structure with an aromatic ring with at least one hydroxyl group, which provides the ability to neutralize reactive species, helping the body to protect itself from oxidative stress (Wojdyla, Oszmianski, & Laskowski, 2009). Additionally, phenols contribute to fruits’ color and taste and have been described as possessing anticarcinogenic and antimutagenic activity (Al-Duais, 2009; Gorinstein et al., 2009). Various studies have shown that phenolic compounds have high antioxidant potential, resulting in a beneficial effect to human health (Vijaya Kumar Reddy, Sreeramulu, & Raghunath, 2010).

Carotenoids are lipophilic compounds formed by 8 isoprenoid units. They play a very important role in human health and nutrition, recognized as strong antioxidants due to their ability to trap singlet oxygen and eliminate the peroxyl radical (Al-Duais, 2009). Some carotenoids have pro-vitamin A activity (β-carotene, α-carotene, γ-carotene, β-cryptoxanthin) and reduce the risk of cancer and coronary vein disease (Yahia & Ornelas-Paz, 2010). Various in vitro and in vivo studies have shown that carotenoids prevent cardiovascular diseases and impact cell signaling pathways (Stahl & Sies, 2005), in addition to providing protection against some types of cancer (Yuan, Stram, Arakawa, Lee, & Yu, 2003).

Based on their structure, carotenoids are divided into two groups: carotenes (hydrocarbonated link) and xanthophylls—with at least one oxygen molecule. The presence of double-conjugated links gives carotenoids the ability to act like photoprotectors (Tanaka, Sasaki, & Ohmiya, 2008), protecting membrane lipid peroxidation quenching reactive oxygen species (Rivera-Pastrana, Yahia, & Gonzalez-Aguilar, 2010). It has been reported that lycopene protects cells from reactive oxygen species (ROS) by stimulating the production of cellular enzymes such as superoxide dismutase and glutathione S-transferase (Goo et al., 2007).
In several fruits such as papaya, the content of carotenoids increases with ripening (Wall, 2006). Lycopene, β-cryptoxanthin, and β-carotene are the main carotenoids that have been identified in papaya (Marelli de Souza, Silva Ferreira, Paes Chaves, & Lopes Teixeira, 2008). Vitamin C (ascorbic acid) is a hydrophilic vitamin present in fruit. It plays an important role because it is required for several metabolic processes like development of tissues and production of hormones (Puente, Pinto-Muñoz, Castro, & Cortés, 2010) and is also considered a powerful antioxidant reducing oxidative stress (Guoong, Mingjun, Fengwang, & Dong, 2009). In addition, it has been considered a powerful antioxidant reducing oxidative stress (Guoong, Mingjun, Fengwang, & Dong, 2009). In addition, it has been observed that vitamin C can act synergically with other vitamins and hormones (Sancho, Yahia, Martínez-Téllez, & González-Aguilar, 2010). However, the identification and quantification of the most important phytochemicals responsible for the antioxidant activity were not reported. Therefore, the objective of this study was to determine the changes in vitamin C and identify and quantify the main phenols and carotenoids during ripening of “Maradol” papaya fruit (Carica papaya L.).

2. Materials and methods

2.1. Chemicals and solvents

Formic acid, acetonitrile, acetone, n-Hexane, dichloromethane, methanol, Na2S2O3, and anhydrous granular sodium sulfate were purchased from J.T. Baker (Baker Mallinckrodt, Mexico). Diethyl ether, methyl tert-butyl ether (MTBE), lycopene (purity ≥ 90%), β-carotene (purity = 95%) from carrots were from Sigma-Aldrich (St. Louis, MO). Solvents used for chromatography were HPLC grade. Water was bidistilled, and HPLC grade water was obtained by a Milli-Q plus water purification system (Millipore Corp., Bedford, MA).

2.2. Plant material

Papaya fruits (C. papaya L. cv. Maradol) were obtained from a local market in Hermosillo, Sonora, Mexico. Fruits were selected for uniform size, color, level of external ripeness, and divided in four ripeness stages: RS1 represents papaya with yellow area on 0–25% of the skin; RS2 (25–50%); RS3 (50–75%), and RS4 (75–100%); we followed the criteria used previously by Fonscea, Rocha-Leal, Cenci, Cecon, and Bressan-Smith (2003) and Santamaría-Basulto et al. (2009). After selection, fruits were divided in lots of 12 fruit each, and flesh and skin were randomly sampled for chemical analysis of vitamin C, phenols and carotenoids. Samples were freeze-dried and stored at −70 °C until analysis.

2.3. Total carotenoids extraction

Total carotenoids (TC) were determined according to Yahia, Soto-Zamora, Brecht, and Gardea (2007) and Ornelas-Paz, Yahia, and Gardea (2008). Freeze-dried papaya tissue (0.5 g) was homogenized in 10 mL of hexane: dichloromethane (1:1, v/v), using an Ultra Turrax® T25 basic homogenizer (IKA Works, Willimmington, NC) and centrifuged at 9000g for 10 min at 5 °C. Organic phase was separated, and procedure was repeated three times. For alkaline hydrolysis 10 mL of methanolic KOH 40% (1:1, v/v) was added to extracts for 1 h at 50 °C in a stirring bath set at 100 rpm. After saponification, 10 mL of 10% sodium sulfate was added for phase separation and the extracts were left for 1 h in the dark at room temperature. TC quantification was measured on top-phase aliquots in a Beckman DU-65 spectrophotometer at 450 and 470 nm. A calibration curve was performed using β-carotene in hexane as the standard and hexane as the blank. Extracts were evaporated in a Rotovapor® (Büchi Labortechnik AG, Flawil, Switzerland) at 30 °C in a Buchi low-pressure evaporator. Samples were resuspended in 2 mL acetone and filtered through nylon membrane of 0.45 μm of pore size (Millipore Corp., Bedford, MA) and directly injected into the HPLC mass spectrometry system.

2.4. High-performance liquid chromatography (HPLC)

Samples (30 μL) containing carotenoids were automatically injected into an HP 1100 series HPLC system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector. Absorption spectra for the main peaks were recorded at 430, 450, and 470 nm. The HPLC system was equipped with a C30 reversed-phase column (4.6 mm × 150 mm) with a spherical particle size of 3 μm (YMC Inc., Wilmington, NC), which was kept at 15 °C. The mobile phase was composed of methanol (A) and methyl tert-butyl ether (B), and the elution gradient was 0 to 100% (B) in 55 min at a flow rate of 1 mL/min and 15 °C.

2.5. High-performance liquid chromatography-ApCI-mass spectrometry (HPLC-ApCI-MS) analysis

Mass spectra of the main carotenoids were obtained using the chromatographic system described above connected to a HP6210 model time-of-flight (TOF) mass spectrometer (Agilent Technologies Inc., Palo Alto, CA) equipped with an atmospheric pressure chemical ionization (ApCI) interface and MassHunter manager software (Version A.02.01). The ApCI–MS system was operated in the positive ion mode. High-purity nitrogen (99.999%) was used as nebulizing (20 psig) and drying gas at a flow rate of 5.0 L/min. Other ApCI–MS parameters were as follows: drying gas temperature 350 °C and the corona, capillary, fragmentor, and skewer voltages were 4 kV, 200 V, and 60 V, respectively. Carotenoids were identified by comparing their retention time and UV–vis spectra with those obtained with reference standards as well as co-chromatography with added standards and using their mass spectra (m/z 100–800). Quantitative data for all-trans-carotenoids were obtained by calibration curves of known standards. Contents of lycopene and β-carotene were obtained from the calibration curves of pure standards (0.001–0.1 g/L), and their correlation coefficients (r²) were 0.9997 and 0.9996, respectively. Quantification of β-cryptoxanthin was performed using the calibration curve of β-carotene, because of its similarity with the spectrum.

2.6. Total phenols and antioxidant capacity

Total phenols (TP) and antioxidant capacity (AOC) of methanol extracts were measured as TEAC (trolox equivalent antioxidant capacity) and ORAC (oxygen radical absorbing capacity) (Gayoso-García Sancho et al., 2010).

2.7. L-Ascorbic acid (AA) and isoascorbic acid (IAA)

AA and IAA were determined according to Corral-Aguayo, Yahia, Carrillo-Lopez, and Gonzalez-Aguilar (2008). Samples of 0.5 g of freeze-dried powder were homogenized in 10 mL of extraction solution [0.1 M citric acid and 0.05% ethylenediaminetetraacetic acid (EDTA) at pH 2.35–2.40] using an IKA T25 basic homogenizer (IKA Works, Willimmington, NC). Then, the homogenate was centrifuged at 15,000g for 10 min at 2 °C. The supernatant was separated and filtered through Sep-Pak C18 Vac 3 mL cartridge (Waters Co., Milford, CT). The first 5 mL was discarded, and the next 3 mL was analyzed. The cartridge was previously conditioned with 10 mL of ethanol and then with 10 mL of HPLC grade water. The residual water was expelled with air. A total of 3 mL of the sample was collected, and 1 mL (0.832 mg/mL) of 1,2-phenylenediamine prepared in methanol/water (5:95, v/v) was added. The samples were incubated for 37 min in the dark and filtered through a 0.45 μm nylon membrane. Aliquots of
40 μL were injected in a HP 1100 Series HPLC (Hewlett-Packard/Agilent Technologies Co., Palo Alto, CA) with 10 μm Bondapak C18 column (3.9 mm × 300 mm), Sentry, 10 μm Bondapak C18 (3.9 mm × 20 mm) guard column, and diode array detector. The mobile phase consisted of 5 mM hexadecyltrimethylammonium bromide (cectimide) and 50 mM KH₂PO₄ in methanol/water (1:99, v/v) at pH 4.6, and flow rate was 1.5 mL/min. l-Ascorbic acid was monitored at 254 nm, and iso-ascorbic acid was monitored at 261 nm. Calibration curves were prepared from known standards and used for quantification. The concentration range and the correlation coefficients (r²) for the calibration curves were between 0 and 0.8 mg/mL and 0.9998 for ascorbic acid and between 0 and 0.8 mg/mL and 0.9995 for isoascorbic acid.

2.9. Statistical analysis

The statistical significance of differences in phenolic compounds, carotenoids, and vitamin C concentrations in papaya of different ripeness stages was analyzed through an analysis of variance (ANOVA) and the multiple comparisons of means through the Duncan’s test. Statistical differences were considered to be significant (p ≤ 0.05) using the statistical software SAS version 8.0 (SAS Inst. Inc. Cary, NC, USA).

3. Results and discussion

The content of total carotenoids in papaya pulp (C. papaya, cv. Maradol) at different ripeness stages (RS) expressed as mg/100 g FW increased with the level of ripeness of the fruit. The highest values were found in fruit of RS4 (3.27 mg/100 g FW), while the lowest value was for RS1 (0.92 mg/100 g FW) (Fig. 1). During ripening, chlorophyll began to degrade, coinciding with carotenoid synthesis and resulting in a significant increase of yellow–orange color. Some tropical fruits such as mangos have a similar behavior as papaya, where color conferred by carotenoids plays an important role in the fruit acceptability by consumers (Yahia & Ornelas-Paz, 2010). The carotenoid content of “Maradol” papayas obtained in the present study were 2-fold higher than those reported for “Formosa” and “Sunrise” papaya (Melo, de Lima Arroxelas Galvão, & Maciel Sucupira, 2006). These differences could be attributed to agricultural practices, sunlight exposure, production area, stage of ripeness, postharvest handling, and methodology used for analysis (Andersson, Olsson, Johansson, & Rumpunen, 2009; De Rosso & Mercadante, 2005; Ornelas-Paz et al., 2008).

The principal carotenoids in saponified extracts of papaya in different stages of ripeness were identified by comparison of mass spectrometry (MS) fragmentation pattern, retention times and UV–visible maximum absorption and mass spectra profile (m/z) of standard compounds, and they were lycopene, β-carotene, and β-cryptoxanthin (Table 1). A similar profile coincides with previous studies done in papaya (Chandrika, Jansz, Wickramasinghe, & Warnasuriya, 2003; Rivera-Pastrana et al., 2010; Wall, 2006). Chromatographic profiles of the main carotenoids present in saponified papaya extracts, which were obtained by HPLC-DAD, are shown in Fig. 2 and correspond to β-cryptoxanthin with a predominant ion [M+H]⁺ at 553 and yielded ion fragments at m/z 409, 576, 653; β-carotene [M+H]⁺ at 537 and yielded ion fragments at m/z 409, 539, 543, 662, and lycopene ([M+H]⁺ at 537 and yielded ion fragments at m/z 530, 576, 669. Previous studies demonstrated the presence of carotenoids as esters in different fruits.
performed by Kimura, Rodriguez-Amaya, and Yokoyama (1991) in present papaya were found between RS1 and RS4. A study increased from 0.24 mg/100 gDW (in RS1) to 0.5 mg/100 gDW (in RS4). Significant differences (P<0.05) between the three carotenoids present in papaya were found between RS1 and RS4. A study performed by Kimura, Rodriguez-Amaya, and Yokoyama (1991) in "Common", "Solo", "Formosa" and "Tailandia" papaya, reported the effect of agricultural practice on the content of the three main carotenoids of papaya fruit. They found that lycopene increased 2-fold while β-carotene increased 2- to 5-fold with respect to that observed in the present study. Our results coincide with those obtained by Rivera-Pastrana et al. (2010) for lycopene, Corral-Aguayo et al. (2008) for β-carotene and those of Wall (2006), who performed studies on the contents of β-carotoxanthin papaya produced under different farming systems, pointing out that the differences could be attributed to the type of farming and the location. The positive health benefits of lycopene contained in different fruits and vegetables have been widely reported, including reduction of cardiovascular problems (Singh & Goyal, 2008). Therefore, the possible benefits of papaya consumption fruit could be compared with those reported in other vegetables rich in lycopene such as tomato. However, the concentration of other phytochemicals present in these products that contribute to health needs to be considered.

Phenolic compounds were also identified in saponified and non-saponified extracts of papaya skin by HPLC-ESI-MS. The major phenolic compounds identified in saponified extracts were hydroxyceamic acid sugar derivatives, while the non-saponified extracts showed only traces of these compounds in an acylated form (data not shown). Caffeic acid was identified tentatively as a [M-H]-deprotonated molecule (m/z 179), with loss of the CO2 group in the form of negative ion, with an UV spectrum (λmax = 280, 320 nm) in a retention time (RT) of 16.8 min (Table 2). p-Coumaric acid was tentatively identified as a [M-H]-deprotonated molecule (m/z 163) in a RT of 19.04 min and yielded ion fragments at m/z 119 and 153. Ferulic acid was tentatively identified according to its UV spectrum (λmax = 280, 320 nm) as a [M-H]-deprotonated molecule (m/z 193), with a RT of 22.4 min, and yielded ion fragments at m/z 117, 134, 149, and 179. Profile of phenolic compounds but not concentration coincides with the first report on the identification of phenolics in "Maradol" papaya skin made by Rivera-Pastrana et al. (2010). Phenolic compounds have been reported to have antiradical, antimutagenic, and anticarcinogen properties and protect plants from UV radiation (Cantin, Moreno, & Gogorcena, 2009;)

(Ornelas-Paz et al., 2008). Papaya extracts were saponified for the hydrolysis of esters and to remove chlorophyll and non-desired fatty acids that could interfere in the analysis. Our results coincided with those of Rivera-Pastrana et al. (2010), who reported a similar profile of carotenoids in “Maradol” papaya obtained from the same production area that the fruits of this study. Marelli de Souza et al. (2008) observed that the major carotenoids found in papaya were lycopene, β-cryptoxanthin, and β-carotene, with lycopene representing 65% of the total. Andersson et al. (2009) observed that the content of esterified carotenoids in cherries increased during ripening, which allows esterified carotenoids to integrate more quickly to the membranes, increasing the color of the fruit and its accumulation in chromoplasts (Yahia & Ornelas-Paz, 2010).

The concentrations of the main saponified and non-saponified carotenoids found in papaya pulp expressed in mg/100 gDW are shown in Fig. 3. The content of lycopene increased 10 times during ripening, showing that RS1 had 0.35 mg/gDW while RS4 had 3.5 mg/100 gDW (Fig. 3A) in the saponified samples. However, this increase was lower in β-cryptoxanthin and increased from 0.25 mg/100 gDW (in RS1) to 1.06 mg/100 gDW (in RS4) and was the second most abundant carotenoid in papaya, followed by β-carotene, which increased from 0.24 mg/100 gDW (in RS1) to 0.5 mg/100 gDW (in RS4). Significant differences (P<0.05) between the three carotenoids present in papaya were found between RS1 and RS4. A study performed by Kimura, Rodriguez-Amaya, and Yokoyama (1991) in “Common”, “Solo”, “Formosa” and “Tailandia” papaya, reported the

**Table 1**

<table>
<thead>
<tr>
<th>Compound name</th>
<th>RT (min)</th>
<th>[M+H]+ (Frag. m/z)</th>
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</thead>
<tbody>
<tr>
<td>β-Cryptoxanthin</td>
<td>14.130</td>
<td>553 (409, 576, 653)</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>16.112</td>
<td>537 (409, 539, 543, 662)</td>
</tr>
<tr>
<td>Lycopene</td>
<td>25.134</td>
<td>537 (530, 576, 669)</td>
</tr>
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**Fig. 2.** HPLC carotenoids chromatograms at 450 nm in papaya flesh (Carica papaya L. cv. Maradol) in four stages of ripeness. RS1 (0–25% yellow), RS2 (>25 and 50% yellow), RS3 (>50 and 75% yellow), and RS4 (>75 and 100% yellow).
Ferulic acid synthesis occurs from phenylalanine via the shikimate (Barone, Calabrese, & Mancuso, 2009). Several studies have indicated anticarcinogenic mechanisms of phenolic compounds. Kawabata et al. (2000) found that ferulic acid had anticarcinogenic effect on colon cancer in rats, and this was correlated with the ability to scavenge free radicals and stimulate the cytoprotective effect of various enzymes. Caffeic acid found in many fruits, vegetables, and coffee is commonly found as esterified form with quinic acid, also known as chlorogenic acid (Clifford, 1999). Da Cunha et al. (2004) observed that caffeic acid and its derivatives exert anti-inflammatory activity both in vitro and in vivo, and this activity was due in part to the removal of nitric oxide (NO) and its ability to modulate the expression of iNOS (inducible nitric oxide synthase). p-Coumaric acid is an intermediate in the synthesis of phenylpropanoids and has been shown to have antioxidant properties, lowers cholesterol, and provides a defense mechanism against atherosclerosis. Zang et al. (2000) found that p-coumaric acid oral administration (317 mg/day for 30 days) inhibited the oxidation of LDL, reduced serum cholesterol levels and did not affect HDL levels, and contributed considerably to the antioxidant capacity, which is directly related to the removal of ROS.

Table 2
Identification of principal phenolic acids by HPLC-ESI-MS in papaya skin (Carica papaya L. cv. Maradol).

<table>
<thead>
<tr>
<th>Compound name</th>
<th>RT (min)</th>
<th>[M − H] − (Frag. m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>16.808</td>
<td>179 (135)</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>19.037</td>
<td>163 (119, 153)</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>22.413</td>
<td>193 (134, 117, 149, 179)</td>
</tr>
</tbody>
</table>

Fig. 3. Carotenoids concentrations in papaya flesh (Carica papaya L. cv. Maradol) saponified and non-saponified by HPLC in four stages of ripeness: Lycopene (A), β-Cryptoxanthin (B), and β-Carotene (C). Data shown are means of at least three determinations and vertical bars represent standard deviation expressed as mg/100 gDW.

Fig. 4. Principal phenolic acids in papaya skin (Carica papaya L. cv. Maradol) in four stages of ripeness. Data shown are means of at least three determinations in HPLC-DAD at 320 nm and vertical bars represent standard deviation expressed as mg/100 gDW.

Fig. 5. Vitamin C (L-ascorbic acid + Isoascorbic acid) in papaya flesh (A) and skin (B) (Carica papaya L. cv. Maradol) in four stages of ripeness. 1, 0–25% yellow; 2, >25 and 50% yellow; 3, >50 and 75% yellow; and 4, >75 and 100% yellow. Data shown are means of at least three determinations and vertical bars represent standard deviation expressed as mg/100 gDW.
The major phenolic acids quantified by HPLC-DAD in papaya skin were ferulic, p-coumaric, and caffeic acids. The contents of phenolic acids decreased concomitantly with fruit ripening (Fig. 4). Ferulic acid had a concentration of 277.49 mg/100 gDW in RS1, decreased to 186.63 mg/100 gDW in RS4; p-coumaric acid showed a concentration of 229.58 mg/100 gDW in RS1 and 135.64 mg/100 gDW in RS4, while the concentration of caffeic acid was 175.50 mg/100 gDW in RS1 and 112.88 mg/100 gDW in RS4. Studies performed in other fruits have determined that hydroxycinnamic acids are generally more abundant than hydroxybenzoic acids, and fruit skin normally has higher concentration of phenolic compounds than that of the pulp (Castillo-Muñoz, Fernández-González, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2009; Gancel, Alter, Dhuique-Mayer, Rualesand, & Vaillant, 2008; Rivera-Pastrana et al., 2010). It has been observed that phenolic compounds decreased during ripening (El Gharras, 2009). The health benefits of phenol compounds are associated with their role in the prevention of several disorders, related to the damaging effect of oxygen free radicals and ROS, (Valko et al., 2007). Liu (2004) found that ferulic acid is covalently conjugated to polysaccharides present in the cell wall, lignin, glycoproteins, and insoluble carbohydrate biopolymers. Among the antioxidant and anti-inflammatory properties that have ferulic acid, it has been observed that it has positive effects on Alzheimer’s disease. Yan et al. (2001) observed that mice with this disease treated with a diet rich in ferulic acid decreased the activity of choline acetyltransferase, mainly due to the electron donation of its 3-methoxy and 4-hydroxyl groups on the benzene ring (Itagaki et al., 2009). Several epidemiological studies suggest an inverse relationship between consumption of foods rich in phenolic acids and the incidence of various diseases. Chung, Moon, Chang et al. (2004) found that caffeic acid and caffeic acid phenethyl ester supply significantly reduced liver metastasis, confirming the anti-tumor and anti-metastatic effects of these phenols. Vitamin C, measured as l-ascorbic and isoascorbic acid and determined by HPLC at 261 nm, both in the skin and pulp was higher in pulp than in the skin, and the greater the stage of ripeness, the greater the vitamin C contents (Fig. 5). Significant differences \( (P<0.05) \) were found in vitamin C between the different RS. The largest amount of vitamin C in the pulp was 58.6 mg/100 gDW (in RS4), and the minimum was 25.1 mg/100 gDW (in RS1). However, skin had lower vitamin C values with 7.4 mg/100 gDW (in RS1) and 23.4 mg/100 gDW (in RS4). These results obtained in the pulp coincide with those obtained by Wall (2006) in eight varieties of papaya, where an average of 51.2 mg/100 g was reported. Corral-Aguayo et al. (2008) obtained 56.2 mg/100 g while Marelli de Souza et al. (2008) reported an average of 75.9 mg/100 g in three papaya cultivars. The content of vitamin C could vary, mainly because of the

**Fig. 6.** Correlation of total carotenoids data in flesh and antioxidant capacity by (A) DPPH, (C) TEAC, and (E) ORAC assay. Correlation of total phenols data in skin and antioxidant capacity by (A) DPPH, (B) TEAC, and (C) ORAC assay.
type of fruit cultivation, type of soil, weather, and level of fruit ripeness (Lee & Kader, 2000). Vitamin C or ascorbic acid is a powerful water-soluble antioxidant that protects the body against oxidative stress, because of its ability to trap hydroxyl and superoxide radicals. In addition, vitamin C is also involved in the synthesis of collagen (Ondrizolka-Serrano, Soliva-Fortuny, & Martin-Belloso, 2008) and regenerates α-tocopherol by reducing α-tocopherol radical (Niki, Noguchi, Tsuchihashi, & Gotoh, 1995). L-Ascorbic acid functions as a protective antioxidant for reactions that require reduced iron (Fe²⁺) or copper (Cu¹⁺) metalloenzymes (Vasdev, Ford, Parai, Longerich, & Gadag, 2001), and a regular daily intake of 250–500 mg reduces oxidative damage by removing free radicals (Tariq, 2007).

An analysis was done on the correlation between the techniques to measure AOC and antioxidant compounds, where DPPH (2, 2-diphenyl-1-picyryldihydrayl) assay showed an elevated correlation with carotenoids in the pulp (R² = 0.9883) (Fig. 6A), with phenolic compounds in the skin (R² = 0.9648) (Fig. 6B), and with ascorbic acid in the pulp (R² = 0.9951) (Fig. 7A). Similar results have been obtained in different types of fruits (Kevers et al., 2007). TEAC (trolox equivalent antioxidant capacity) assay showed a high correlation between carotenoids in the pulp (R² = 0.9804) (Fig. 6C), phenols in the skin (R² = 0.9651) (Fig. 6D), and ascorbic acid in the pulp (R² = 0.9598) (Fig. 7B). On the other hand, ORAC (Oxygen Radical Absorbing Capacity) assay also showed high correlation with carotenoids (R² = 0.9738) (Fig. 6E), phenols (R² = 0.9930) (Fig. 6F), and ascorbic acid (R² = 0.9804) (Fig. 7C). These results coincide with those obtained by Corral-Aguayo et al. (2008) and Guorong et al. (2009) but differ from the results obtained by others (Mahattanataweewee et al., 2006), where the correlation of ascorbic acid with ORAC was minimum (R² = 0.23). These differences are perhaps due to the types of fruit cultivation and procedures used in the extraction of the sample (Cantin et al., 2009; Corral-Aguayo et al., 2008).

4. Conclusions

Results indicate that ferulic, p-coumaric, and caffeic acids are the most abundant acids in papaya skin. The most abundant carotenoids in pulp are lycopene, β-cryptoxanthin, and β-carotene. Vitamin C contents were higher in the pulp than in the skin, showing an increase of 233.7% in RS4 with respect to RS1. On the other hand, concentrations of phenolic compounds, carotenoids, and vitamin C were highly correlated with the antioxidant capacity measured by DPPH, TEAC, and ORAC. Results indicate that the consumption of ripened papaya is better due their higher concentrations of bioactive compounds, which can contribute to improve human health. As far as we know, this is the first report on “Maradol” papaya that evaluates the effect of RS on the changes of the most important phytochemical compounds. This information can be useful in determining the possible role of the identified compounds that can participate in the prevention of different health disorders. Further studies are needed to evaluate the biodisponibility of phytochemicals present in papaya, after being consumed fresh or processed.

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