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Effect of ripening on physico-chemical properties and bioactive compounds in papaya pulp, skin and seeds

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Byproducts generated by the food industry represent an alternative to obtain functional ingredients. Byproducts of tropical fruits, such as papaya skin and seeds, represent a source of bioactive compounds (BC), which could change during fruit ripening. Effect of ripening stage (RS) on BC content and antioxidant properties of edible pulp, skin and seeds of papaya cv. Maradol was determined. Papaya skin showed significantly higher ascorbic acid (~250 mg AAE/100 g) content than seeds (~20 mg/100 g), while pulp had the highest values (~600 mg/100 g). However, papaya skin presented higher total phenolic content (~560 mg GAE/100 g) and flavonoids (~1000 mg QE/100 g) than pulp and seeds. Also, papaya skin showed the highest values followed by pulp and seeds with TEAC, FRAP and DPPH. Papaya skin had higher carotenoids and α-tocopherol (~1500 µg/100 g and ~4000 µg/100 g, respectively) content than pulp and seeds. BC content in each byproduct varied in all RS. Therefore, among the papaya byproducts, skin represents a good source of BC with good antioxidant properties, which may be used to extract them for its incorporation in functional foods depending on RS.

Keywords: Antioxidant capacity, Bioactive compounds, Byproducts, Papaya, Ripening stage.

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Introduction

Consumption of tropical fruits has been increasing in national and international markets due to their nutritional and therapeutic properties. Most of the time, tropical fruits are processed to separate desired value products from other plant tissues, which results in the generation of byproducts such as skin, seeds and non-edible pulp[1]. Disposal of these byproducts represents a serious problem related to waste handling, environmental considerations, legal restrictions and economical limitations[2]. Currently, an alternative to decrease this problem is to use such byproducts in the design and development of functional foods or nutraceutical ingredients. Byproducts represent a source of bioactive compounds (BC) such as dietary fibre, vitamin C, phenolic compounds, flavonoids and carotenoids which are related to health benefits due to their antioxidant properties. The content of such BC may vary according to pre and postharvest conditions, ripening stage (RS), type of tropical fruit and byproduct used[3]. Furthermore, since byproducts still have BC, their characterization and extraction is an important target for the pharmaceutical, cosmetic and food industries, considering their biological properties. Use of entire fruit tissues is important because it could bring economic benefits to producers and a beneficial impact on the environment[4].

Among the tropical fruits, papaya (*Carica papaya* L.) is widely cultivated in tropical countries for its edible fruit, and also used for some people in the traditional medicine. Despite this, many countries such as USA, Australia and European Union do not use the papaya byproducts[3]. During papaya processing, 6.51 % of seeds, 8.47 % of skin, 32 % of unusable pulp, and 52.96 % of the final product are produced[5]. Generation of such waste material suggests papaya producers would benefit greatly by finding added value for all these byproducts. Characterization of

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different parts of ripe and unripe papaya, as well as their leaves and seeds, is a good way to indicate that its whole utilization may supply an important amount of necessary nutrients such as vitamins and BC[3]. The main BC reported in papaya pulp, skin and seeds are carotenoids, phenolic acids and flavonoids, among others[1,3,6]. Due that BC has been related with biological properties, papaya byproducts could be used as antimicrobial, antioxidants, colourants, flavouring, thickening agents, and others[1]. Then, the BC present in edible papaya and its byproducts makes them interesting ingredients for the food industry, especially in the design of functional foods or nutraceutical ingredients.

It is worth mentioning the importance of BC from fruits and vegetables is related to their antioxidant properties, which have been correlated with degenerative disease prevention[7]. The aim of this work was to analyze the effect of the RS on the BC content and antioxidant properties of edible pulp, skin and seeds of papaya cv. Maradol.

Materials and Methods

Material

Papaya fruits (*C. papaya* L. cv. Maradol) were obtained from a local market in Hermosillo, Sonora, Mexico. Fruits were selected for uniform size, colour, and degree of external ripeness. Fruits were classified in four RS according to yellow area percentage on their skin: RS I (0-25 %), RS II (25-50 %), RS III (50-75 %), and RS IV (75-100 %) as reported by Sancho *et al*[6] (Table 1). Fruits were immersed in a 200 ppm sodium hypochlorite solution for 2 min, drained, and dried at room temperature for 1 hour. Four fruits were prepared to analyze their physico-chemical properties. On the other hand, the skin, seeds and pulp (tinny slices) of 4 fruits were freeze-dried and ground under red light conditions. The yield of byproducts (skin and seeds) before freeze-drying was determined. To avoid oxidation of the BC, ground samples were stored at -36 ˚C until analysis.

Physico-chemical properties

Colour and firmness

The CIE L*, a* and b* parameters were measured in four parts of the pulp and skin of papaya with a colourimeter Minolta CR-300 (Konica, Minolta, Sensing, USA). The Hue angle and Chroma values were calculated as follows: Chroma = $(a^{*2}+b^{*2})^{1/2}$ and Hue angle = $\tan^{-1}(b^*/a^*)$ as was reported by Odriozola-Serrano, Soliva-Fortuny, Martín-Belloso[8]. Pulp firmness was measured at six points close to the peduncle, the centre and the apex on opposite sides of papaya fruit using a penetrometer (Chantillon DFM50) fitted with an 8 mm wide and flat-end probe (stainless steel). Results at each RS were obtained from four different papayas and expressed as the force (N) needed to penetrate the fruits.

Total soluble solids, pH and titratable acidity

A sample (10 g) was blended with 60 mL of distilled water (pH 7) and filtered. The supernatant was analyzed directly with a digital refractometer (Palette Digital PR-10, Atago, Japan) to obtain the total soluble solids (TSS, ˚Brix). About 50 mL of supernatant was used to measure the pH with an automatic titrator (DL28 Titrator, Mettler Toledo, USA). Titratable acidity (TA) was determined by AOAC 942.15 method[9], samples were titrated against 0.1 N NaOH until pH 8.2 was achieved. The analysis was done in quadruplicate and reported as grams of citric acid/100 g of fresh weight (g citric acid/100 g FW).

Hydrophilic compounds

Ascorbic acid

Ascorbic acid extraction was done according to Doner[10]. Sample (0.5 g) was well mixed with 3 % metaphosphoric acid solution (10 mL) and centrifuged (3000 g, 10 min, 4 ˚C). The supernatant was filtered with a 0.22 μm nylon filter before analysis. A Waters μBondapak NH_2 analytical column (3.9x300 mm, 10 μm, 125 A, Waters Co. Milford, MA) fitted with the same guard column was used for separation. Samples were run using a Dionex Ultimate 3000 ultra high performance liquid chromatography equipped with an Accela 600 pump (Thermo Scientific, San Jose California, USA). An Accela photodiode array (PDA) detector set at 268 nm and PC with Chromeleon 6.8 chromatography data system (Thermo Scientific, San Jose California, USA) were used for control and integration. An isocratic method was used to analyze the samples with filtered acetonitrile: 0.05 M KH_2PO_4 (75:25, v/v) as the mobile phase. The flow rate was 1 mL/min, injection volume was 20 μL. Ascorbic acid was identified by comparing its retention time with that of a standard, results were expressed as mg ascorbic acid equivalents/ 100 g of dry weight (mg AAE/100 g DW). Assays were done in triplicate and under red light conditions to avoid ascorbic acid oxidation.

Table 1 — Color parameters of papaya pulp and skin in different ripening stage.

Vegetable tissue		Ripening stage	L*	a*	b*	˚Hue	Chroma
Pulp		I	47.19[a]	12.27[a]	21.11[a]	60.70[a]	24.76[a]
		II	49.20[a]	21.27[b]	38.16[b]	61.00[a]	43.78[b]
		III	57.74[b]	21.94[b]	41.93[c]	62.38[a]	47.36[c]
		IV	57.66[b]	19.98[b]	43.43[c]	65.25[a]	47.84[c]
Skin		I	46.32[a]	-14.37[a]	33.13[a]	114.51[c]	36.49[a]
		II	52.91[b]	-6.37[b]	43.68[b]	98.78[b]	44.46[b]
		III	62.07[c]	1.46[c]	55.16[c]	88.90[a]	55.36[c]
		IV	63.08[c]	5.83[c]	58.26[c]	84.49[a]	58.76[c]

Data represent the means of n= 4. Values with the same letter in the same column for each vegetable tissue are not significantly different (p <0.05).

Sample extraction

Sample extraction for total phenolic compounds (TPC), total flavonoids content (TFC) and antioxidant capacity (AOXC) was performed in triplicate using a method described previously[11]. Dried samples (1 g) were extracted with 10 mL 80 % methanol and sonicated for 30 min (Bransonic Ultrasonic Co., Model 2210, Danbury, USA). After centrifugation (14000 rpm, 15 min, 4 ˚C), supernatants were collected and filtered; the precipitate was washed twice under described conditions. Supernatants were combined up to a final volume of 50 mL and stored at -20 ˚C until analysis.

Total phenolic compounds

TPC was determined according to the Folin-Ciocalteu method. The methanolic extract (30 µL) was put in a microplate and 150 µL of Folin-Ciocalteu reagent was

added. After reacting for 3 min, 120 µL of 7.5 % Na_2CO_3 solution were added to start the reaction. Samples were incubated for 20 min at room temperature under subdued lighting conditions. Absorbance was measured at 765 nm in a microplate reader[11]. Results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry weight (mg GAE/100 g DW).

Total flavonoids content

The TFC was measured following the method described by Quirós-Sauceda *et al*[11]. Briefly, 100 µL of the methanolic extract was mixed with 430 µL of 5 % $NaNO_2$ solution. After 5 min, 30 µL of 10 % $AlCl_3 \bullet 6H_2O$ solution was added and allowed to stand for 1 min. Finally, 440 µL of 1 M NaOH was added and the sample was mixed well by vortexing. Absorbance was measured at 496 nm using a microplate reader (FLUOstar Omega, BMG LABTECH, Durham, USA). TFC were calculated using a quercetin standard curve, results are expressed as mg quercetin equivalents/100 g of dry weight (mg QE/100 g DW).

Antioxidant capacity

AOXC of papaya pulp, skin and seed methanolic extracts was measured by the trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP) and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity[11].

TEAC assay: Briefly, ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mmol/L ABTS stock solution with 2.45 mmol/L potassium persulphate in the dark at room temperature for 12-16 h before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70±0.02 at 750 nm. The reaction was conducted in a microplate with the addition of 245 µL of $ABTS^{\bullet+}$ solution to 5 µL of methanolic extract. After 5 min, the absorbance of the samples was measured in a microplate reader and the results were expressed as mg trolox equivalent per 100 g of dry weight (mg TE/ 100 g DW).

FRAP assay: FRAP working solution was freshly prepared by mixing well 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 20 mM $FeCl_3$ solution and 2.5 mL of 10 mM TPTZ solution in 40 mM HCl. To perform the assay, 20 µL of methanolic extract was placed in a microplate well and mixed with 280 µL of FRAP working solution. Samples were incubated at room temperature for 30 min in the dark, their absorbance was measured at 630 nm. Results were expressed as mg trolox equivalent per 100 g of dry weight (mg TE/100 g DW).

DPPH assay: A DPPH radical solution was prepared by dissolving 2.5 mg of DPPH radical in 100 mL of methanol. The absorbance of the radical solution was adjusted to 0.70±0.02 at 516 nm. Aliquots of 20 µL of sample were placed in a microplate well and 280 µL of DPPH radical was added. Samples were kept at room temperature for 30 min in the dark, and their absorbance was measured with a microplate reader. Results were reported as mg trolox equivalent per 100 g of dry weight (mg TE/100 g DW).

Lipophilic compounds

Carotenoids and α-tocopherol were extracted with methanol and tetrahydrofuran, dried under nitrogen and analyzed by HPLC as previously described by Riso and Porrini[12]. After extraction was performed, the sample was dissolved in 1000 µL of ethanol, filtered and injected (20 µL) into a C30 YMC-Carotenoid S-3 (3 mm, 150x4.6 mm, Milford, MA) HPLC column. HPLC system consisted of Agilent 1220 separation module with photodiode array detector and ChemStation software (Agilent Technologies, Inc., Santa Clara, CA). Absorbance was monitored at 290 nm and 450 nm for α-tocopherol and carotenoids quantification, respectively. The concentration of carotenoids and α-tocopherol was calculated using an external calibration curve derived from pure standards (α-tocopherol, α-carotene, all-trans-β-carotene, cryptoxanthin, lutein, and zeaxanthin) obtained from Sigma-Aldrich (St. Louis, MO).

Statistical analysis

Results are expressed as mean±standard deviation in all analysis except for lipophilic compounds, which were detected once. One-way analysis of variance (ANOVA) was carried out using the statistical software JMP, version 7.0. Significant differences (p <0.05) among ripening stage for each by product were performed by Tukey HSD multiple comparison test.

Results and Discussion

Colour development of pulp and skin from papaya

Colour development is commonly measured to evaluate the RS in fruits. Table 1 shows colour parameters (L*, a*, b*, °Hue and Chroma) of papaya pulp and skin. Papaya pulp presented significant differences (p <0.05) between RS I and IV in all

parameters, except ˚Hue values. The same trend was observed in papaya skin, where the higher the RS, the higher the colour parameters, except ˚Hue values which decreased during the fruit ripening. The colour L* and a* values of pulp and skin increased from RS I (47.19 and 12.27) to III (57.74 and 21.94), while at RS IV this parameter was not different to that of RS III in each papaya tissue. According to Basulto *et al*[13], the increment of L* and a* values in papaya skin during ripening are due to changes from green to red colour. In this case, negative a* values represent a green colour, while positive values indicate red. On the other hand, yellow colour changes during fruit ripening were observed in both pulp and skin according to the colour b* values in each vegetable tissue. The higher b* values indicated yellowness during fruit ripening (Table 1). Similar results were reported in papaya cv. Maradol[13,14].

˚Hue values of papaya pulp during ripening did not show significant differences (p <0.05) (Table 1). Contrary to the pulp, increasing RS correlated with lower ˚Hue value in papaya skin from RS I (114.51) to IV (84.49). A decrease of ˚Hue values indicates colour changes from green to orange in the skin, while, in pulp, this change means an increase in orange colour as was observed in L*, a* and b* values. Chroma value in both papaya pulp and skin increased with the RS and is related to a diminution of colour saturation. Chroma values showed significant differences (p <0.05) with RS, varying from 24.76 to 47.84 in pulp and from 36.49 to 58.76 in the skin. Similar results were reported in papaya cv. Maradol, Costa Rican hybrid "Pococí" and papaya cv. Eksotika[13-16].

Ripening of fruits usually starts together with an increase of fruit respiration. In the case of climacteric fruits as papaya, during ripening, there is an increase in their respiration rate and ethylene biosynthesis. Ethylene production causes physicochemical and biochemical changes on the fruit due to the activation of enzymes responsible for these events[17]. Observed colour changes during papaya ripening are attributed to biochemical processes such as chlorophyll degradation and carotenoids biosynthesis, which result in the increase of yellow-orange colour in papaya[6]. Ornelas-Paz *et al*.[18] reported that the increase in yellow-orange intensity on mango pulp and skin was accompanied with a rise of the a*, b* and Chroma values, and a reduction in the L* and ˚Hue values. The authors correlated these colour changes with an increase in carotenoids content. However, according to the authors, such correlation was strong in 'Manila' mango compared to 'Ataulfo' mango due to the composition of xanthophylls in each mango cultivar. In the case of papaya, β-carotene, xanthophylls as lycopene and β-cryptoxanthin have been reported, which may influence colour parameters in these fruits compared to mango[6,19]. Furthermore, colour changes in papaya pulp and skin are associated to RS and biochemical changes, indicating chlorophyll loss, synthesis of coloured pigments (carotenoids) and unmasking others formed before fruit ripening[16]. All of these factors can influence the bioactive compounds content and the fruit quality as firmness, TTS, TA, etc.

Physico-chemical properties of papaya pulp

During papaya ripening, changes in the physicochemical properties have been attributed to physiological, biochemical and molecular events which affect its nutritional and quality properties. During ripening, macromolecules such as starch or dietary fibre start to break down into low molecular weight molecules such as sugars, organic acids, among others, causing changes on the physicochemical properties of papaya[14]. Table 2 shows the physico-chemical properties of papaya pulp at different RS. As expected, TSS of papaya pulp increased during ripening, reaching a final value of 9.72 (RS IV) which represented an increase of 20 % respect to the RS I. On the other hand, it was observed that there was an inverse correlation between RS firmness of papaya pulp (p <0.05). However, pH and TA did not change

Table 2 — Total soluble solids (TSS), pH, titratable acidity (TA) and pulp firmness of papaya at different ripening stage.

Ripening stage	TSS	pH	TA (g Citric acid/100 g FW)	Firmness (N)
I	7.80±0.27^{a}	5.54±0.09^{a}	4.51±0.06^{a}	126.63±2.54^{d}
II	8.16±0.59^{a}	5.87±.0.05^{b}	4.42±0.03^{a}	33.80±1.03^{c}
III	8.88±0.22^{a}	5.80±0.03^{b}	4.86±0.07^{a}	21.72±0.60^{b}
IV	9.72±0.22^{b}	5.99±0.06^{b}	4.64±0.20^{a}	15.00±0.27^{a}

Data represent the mean±standard error, n= 4, fresh weight (FW). Values with the same letter in the same column are not significantly different (p <0.05).

for different RS. In previous studies, TSS increased with RS due to hydrolysis of cell wall components such as pectin and hemicellulose, which results in the accumulation of carbohydrates of low molecular weight[15]. Also, TSS increased with hydrolysis of starch as papaya fruit is ripening[20]. These changes were correlated with the firmness decrease of papaya pulp. According to Schweiggert *et al.*[15], firmness loss occurs because there is an increase of the polygalacturonase (PG) and β-galactosidase activity, which are responsible for breaking down pectin and hemicelluloses. Sancho *et al.*[14] in the same papaya cultivar of this study reported an increment in PG and pectinmethylesterase (PME) activity during ripening. The authors mention PG is responsible for the softening during ripening due to pectin's deesterification, while PME removes metoxyl groups from small ramifications of pectin or partially esterified homogalacturonates, providing a substrate for the PG activity, and modifying the pH from the cell wall.

Since pH and TA were similar among RS, TSS and firmness values are considered good parameters to evaluate the ripening index of papaya and its quality. TSS and firmness values in this research are in agreement with those reported for other papaya varieties[13,15,16,20]. However, pH and TA showed slight differences with respect to literature, which may be attributed to papaya varieties, agronomical and environmental conditions and postharvest treatments. As mentioned above, PG and PME activities in different RS affect the physicochemical properties of papaya fruit, and it can depend on the respiration rate and ethylene production during ripening.

Papaya pulp, skin and seeds yield

During papaya consumption, a certain amount of peel and seeds are generated as a waste material[5]. However, some reports mention that these byproducts are a source of BC which can be identified, extracted and used as food ingredients. For that reason, the yield (%) of edible papaya pulp, skin and seeds for each RS is reported in Table 3**.** No significant differences in pulp and skin yield from papaya were observed among RS. However, seed yield showed significant differences (p <0.05) between RS I and II (4.15 % and 2.14 %, respectively). These results are low compared to that percentage of recovery reported by Ayala-Zavala *et al.*[5] during fresh-cut papaya process. Such differences could be attributed to the fruit cut processing, because of this industry demand shape uniformity in the final product, which allows generating a high amount of unusable pulp and skin. According to the same authors, pineapple generates 51.96 % of byproducts (between core, peel, unusable pulp and top) more than papaya (47.04 %). In addition, another fruit with high byproducts recovery is mango (42.44 % between seed, peel and unusable pulp).

According to our results, around 11 % of papaya fruit weight corresponding to skin and seed is considered waste and does not have any application. Such waste represents an economic loss to the producer because is an organic residue which needs specific management. However, if the BC of these residues characterized by their antioxidant and functional properties are analyzed, they could gain an added value for the food industry, because they can be used as food ingredients, especially in the design and development of functional foods or nutraceutical ingredients. Therefore, high recovery percentage of byproducts generated after postharvest processing, and the BC characterization and extraction of such material should be analyzed from the economic point of view, cause exploitation of the entire fruit could have economic benefits to the food industry, consumers and environment[1].

Changes of bioactive compounds in papaya pulp, skin and seeds during ripening

Ascorbic acid, TPC and TFC content of papaya pulp, skin and seed in different RS is depicted in Fig. 1**.** Papaya pulp presented a significant increase in ascorbic acid content during ripening (463.03 mg AAE/100 g to 685.68 m AAE/100 g DW). This means higher RS will yield increased ascorbic acid content. Sancho *et al.*[6] also found an increase in ascorbic acid content in the pulp of papaya cv. Maradol. Despite the use of the same papaya variety in both studies, they reported lower ascorbic acid values than our results. Such differences may be due to cultivation and post-

Table 3 — Recovery (%) of pulp, skin and seeds from papaya at different ripening stage.

Ripening stage	Recovery (%)		
	Pulp	Skin	Seed
I	88.27±0.47^{a}	7.67±0.45^{a}	4.15±0.43^{b}
II	90.00±0.43^{a}	7.66±0.35^{a}	2.14±0.67^{a}
III	89.57±0.22^{a}	7.35±0.25^{a}	2.90±0.14^{ab}
IV	88.74±0.65^{a}	8.01±0.30^{a}	3.14±0.40^{ab}

Data represent the mean±standard error, n= 4. Values with the same letter in the same column are not significantly different (p< 0.05).

harvesting practices, soil, environmental conditions, among others. With respect to papaya skin, ascorbic acid content was decreasing from RS I (278.6 mg AAE/100 g DW) to RS III (186.64 mg AAE/100 g DW) and increased again in RS IV (301 mg AAE/ 100 g DW) (Fig. 1). On the other hand, papaya seed presented the lowest ascorbic acid content (around 20 mg AAE/100 g DW) and did not show significant differences among RS (p <0.05). Ascorbic acid content increases during fruit ripening due to ethylene production and can neutralize free radicals generated during the biosynthesis of plant hormones, act as a substrate for oxalate and tartrate biosynthesis, and also can be oxidized during the xanthophyll cycle[17]. As a result, ascorbic acid content in papaya pulp during ripening is high, but, in the case of papaya skin, its content decrease may because this tissue has more protective functions during ripening as mentioned above. Respect to seeds, low ascorbic acid detected could indicate this molecule is used as an antioxidant against reactive oxygen species (ROS) generated during seed birth to death[21]. Importance of ascorbic acid as the main biological active form of vitamin C comes from its nutritional value and

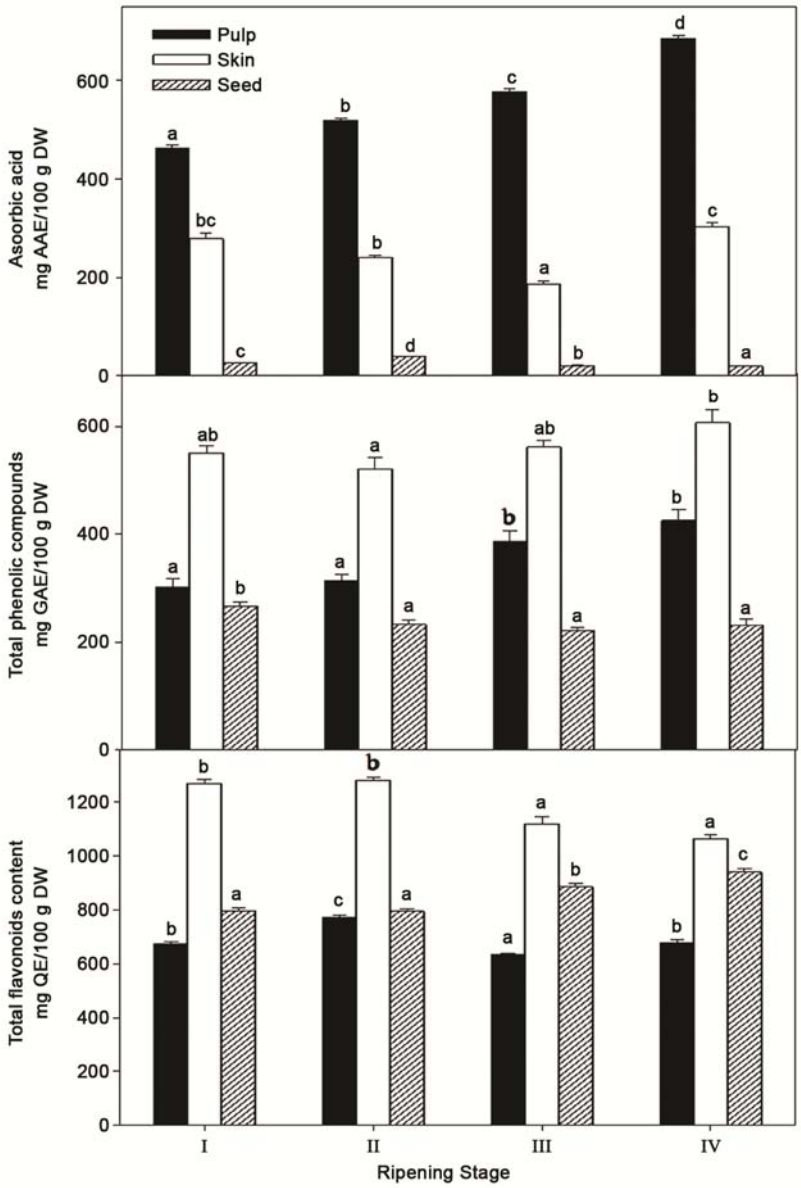

Fig. 1 — Ascorbic acid, total phenolic compounds, and flavonoids content of papaya pulp, skin and seeds in different ripening stage. Data represent the mean±standard deviation (n= 3), dry weight (DW). Values with the same letter for each papaya tissue are not significantly different (p <0.05). GAE: Gallic acid equivalents, QE: Quercetin equivalents.

antioxidant properties because it is essential in posttranslational modifications of collagen and prevents mutation in human DNA, among others[22]. Hence, these results indicate papaya pulp and skin represent a good source of ascorbic acid that can be used by the pharmaceutical and food industry as nutraceutical or food ingredient, respectively.

TPC content was high in papaya skin, followed by pulp and seeds (Fig. 1). During fruit ripening, TPC increased in pulp and skin showing significant differences (p <0.05) among RS. Probably, the presence of ascorbic acid may affect the TPC determination because of the Folin-Ciocalteu reagent is reacting with this molecule and other organic acids[22], leading to an increase of TPC content during ripening as ascorbic acid does. In addition, papaya skin presented the higher TPC content than pulp and seeds because in this part of the fruit there is more polyphenol biosynthesis to protect against solar radiation, acting as an attractant in fruit dispersal, and also as a defence mechanism to different kinds of stress during fruit growth and ripening[23]. According to Villa-Rodríguez *et al.*[24], ethylene production increases the activity of phenylalanine ammonia lyase involved in the biosynthesis of PC, which in consequence increase the TPC during ripening. On the other hand, seeds did not show significant differences and a decrease of TPC concentration from RS I (266.52 mg GAE/ 100 g DW) to RS II (232 mg GAE/ 100 g DW) was observed. Such reduction of TPC could be attributed to the decrease of soluble PC related to the reduction of primary metabolism, causing a deficiency in substrates for its biosynthesis. Moreover, soluble PC in seeds may have been transformed into insoluble PC linked to the cell wall as hydrolysable or condensed tannins[25], which cannot be extracted with methanol. In addition, high protein, oil, and crude fibre content present in papaya seeds could be a factor that decreased the PC biosynthesis in this tissue[26]. However, the presence of ROS during ripening because of their functions in the regulation of the cellular redox state [21], it may decrease the TPC content in seeds together with ascorbic acid.

With regards to flavonoids, skin and seeds were determined to be a good source of TFC and showed higher values compared to those of papaya pulp (Fig. 1). The TFC in papaya skin began to decrease from 1285 mg QE/ 100 g DW (RS I-II) to 1064.5 mg QE/100 g DW (RS III-IV), while in seeds this parameter increased during ripening (797 mg QE/ 100 g DW to 940 mg QE/ 100 g DW). TFC in papaya pulp did not show significant differences among RS (p <0.05). The same trend was observed in avocado pulp during ripening[24]. These results indicate that during papaya ripening flavonoid biosynthesis is slow or maybe these compounds are being hydrolyzed by the ripening effect since they showed an opposed trend to that of TPC content. The TFC increment in seeds during ripening can be attributed to the accumulation of flavanols, anthocyanins, proanthocyanidins and their precursor (-)epicatechin as was reported in *Arabidopsis thaliana*[27]. Such trend is in agreement with the decrease in the TPC, which indicated that free PC is being transformed during ripening into bound PC, or that PC as phenolic acids are being acted as antioxidants together with ascorbic acid against ROS, which decreases its content. Consequently, the importance of TFC based on its antioxidant capacity is due to its ability to decrease the ROS formation and scavenge free radicals[24], as well as because it has been noted that TFC reduces the deteriorative reactions. It means that TFC could be associated with the increment of shelf life as was reported in mango[22].

According to the bioactive compounds determined in papaya skin and seeds, it is possible to observe that skin represents a good source of ascorbic acid, while both of them are a good source of TPC and TFC. However, the optimal yield of each bioactive compound will depend on the RS of the papaya tissue as was observed. Hence, skin and seeds from papaya represent a waste material which can be used to extract, isolate and apply different BC as food ingredients in the development of nutraceutical or functional foods. Such practices are going to have benefits for the producer and the consumer because of the added value that this waste material can have depending on its RS and BC content.

Antioxidant capacity

AOXC measurement of phenolic compounds (PC) in plant foods is important to understand their correlation with human health benefits. Among the different methods to determine AOXC of PC in foods, TEAC, DPPH and FRAP are the most reported. These methods are related to the capacity of the antioxidant to reduce radicals by hydrogen atom transfer (HAT) and single electron transfer mechanisms (SET). FRAP is considered a SET mechanisms, while TEAC and DPPH are mixed assays[28]. Therefore, the AOXC of papaya pulp, skin and seeds in different RS is depicted in Fig. 2. The AOXC by TEAC assay was high in papaya skin (around 1125 mg TE/ 100 g DW) compared to the pulp and seeds, and there were no

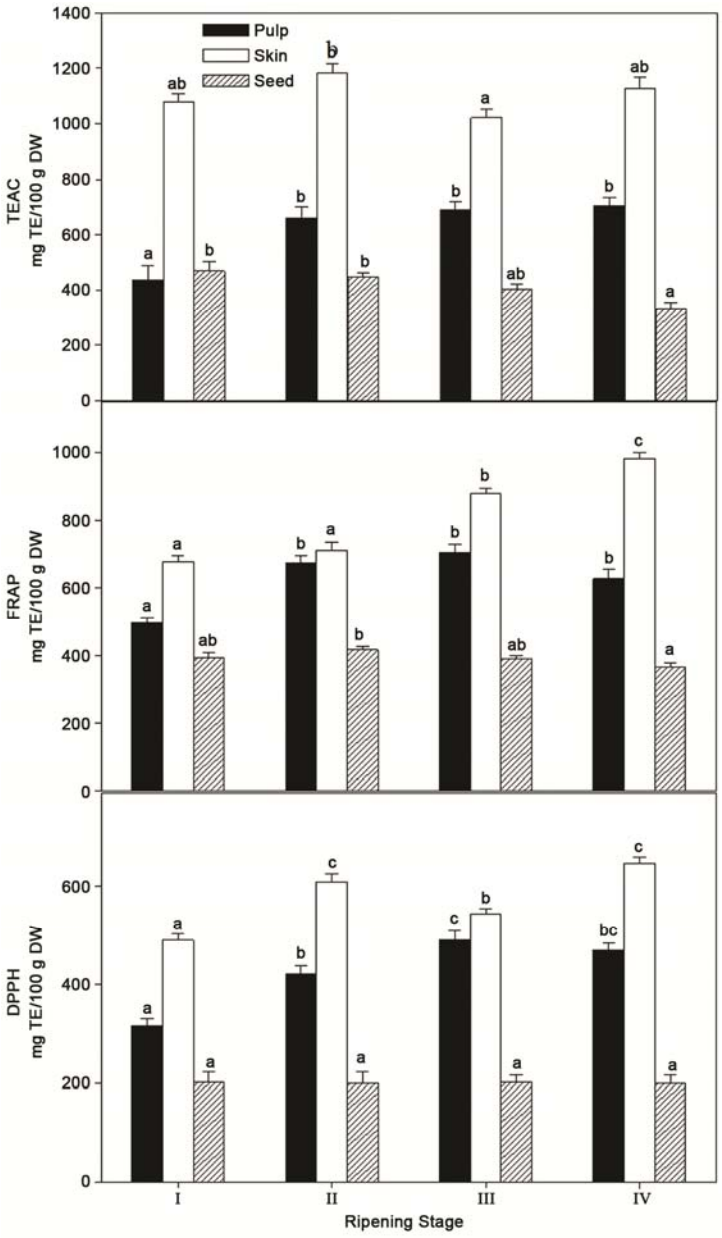

Fig. 2 — Antioxidant capacity of papaya pulp, skin and seeds in different ripening stage. Data represent the mean±standard deviation (n= 3), dry weight (DW). Values with the same letter for each papaya tissue are not significantly different (p <0.05). TE: Trolox equivalents.

observed statistical differences (p <0.05) among RS. In the case of pulp, RS I presented lower AOXC (435.09 mg TE/100 g DW) than RS II-IV (around 691 mg TE/ 100 g DW). This is in agreement with that reported by Zuhair *et al*.[20] in papaya pulp. On the other hand, a slight decrease in the AOXC during the different stages of ripening was observed in papaya seeds without significant differences. It has been reported that $ABTS^{\bullet+}$ reacts with PC and ascorbic acid, while lipophilic extracts make this radical cation weak affecting its capacity to scavenge this radical[20]. Probably, low ascorbic acid and TPC content of seeds decreased AOXC in TEAC assay. Also, papaya seeds are rich in lipophilic compounds which interfere with the capability of the hydrophilic compounds to scavenge the $ABTS^{\bullet+}$ radical cation, as an example, the interaction of phenolic acids with carotenoids can affect the AOXC[29].

The capacity of the samples to reduce the Fe^{3+} to Fe^{2+} using the FRAP assay was determined. Papaya skin showed a tendency to increase the AOXC with the fruit ripening from 678.61 mg TE/ 100 g DW (RS I) to 982.01 mg TE/ 100 g DW (RS IV) (Fig. 2). AOXC of RS II-IV

(around 650 mg TE/ 100 g DW) was higher than RS I (496.58 mg TE/ 100 g DW) in papaya pulp; while AOXC in seeds was similar among RS. FRAP values increment with the pulp ripening process was similar to that reported in the pulp of papaya cv. Hongkong[20]. However, there is not much information about the PC reducing power in papaya seeds. Low FRAP values in seeds compared to pulp and skin could be attributed to the low reducing power of TFC in methanol extracts.

Regarding DPPH scavenging ability of the samples (Fig. 2), higher ripening stage correlated with higher AOXC in papaya skin followed by pulp and seeds. Sancho *et al*[29] found caffeic, ferulic and p-coumaric acids as the main PC in papaya skin. Evaluating AOXC of these PC in combination, they concluded caffeic acid had the greatest AOXC individually followed by ferulic and p-coumaric acid, and radical scavenging ability of the three combined PC decreased. Despite the fact that during papaya skin ripening the concentration of ferulic, caffeic and p-coumaric acids decrease[6], an increase in the AOXC measured by DPPH may be resulting from synergistic interactions among these PC. The increase of AOXC in papaya pulp with RS by DPPH also was reported in papaya cv. Hongkong[20]. In papaya seeds, AOXC did not show changes among RS. Although seeds had a high TFC, their low scavenging ability could be attributed to the low TPC and vitamin C, PC structure, and antagonistic effects occurring during ripening.

In general, papaya skin showed the highest AOXC values in all three assays, while seeds presented the lowest values compared to the pulp. Also, fruit ripening affected the AOXC values in each papaya tissue. According to the results, BC from papaya pulp, skin and seeds can act as antioxidants depending on their chemical structure, the presence of hydroxyl groups in their structure, and interactions between BC as ascorbic acid, or lipophilic compounds as carotenoids, among others. Ascorbic acid, TPC, TFC and AOXC studied along papaya RS can vary because there are a lot of physiological and biochemical changes occurring that needs defence mechanisms to avoid oxidative stress through the activation of enzymes involved in the PC biosynthesis[22].

Lipophilic compounds

Among the lipophilic compounds found in fruits and vegetables are the tocopherols and carotenoids. Fig. 3 shows that skin presented the highest

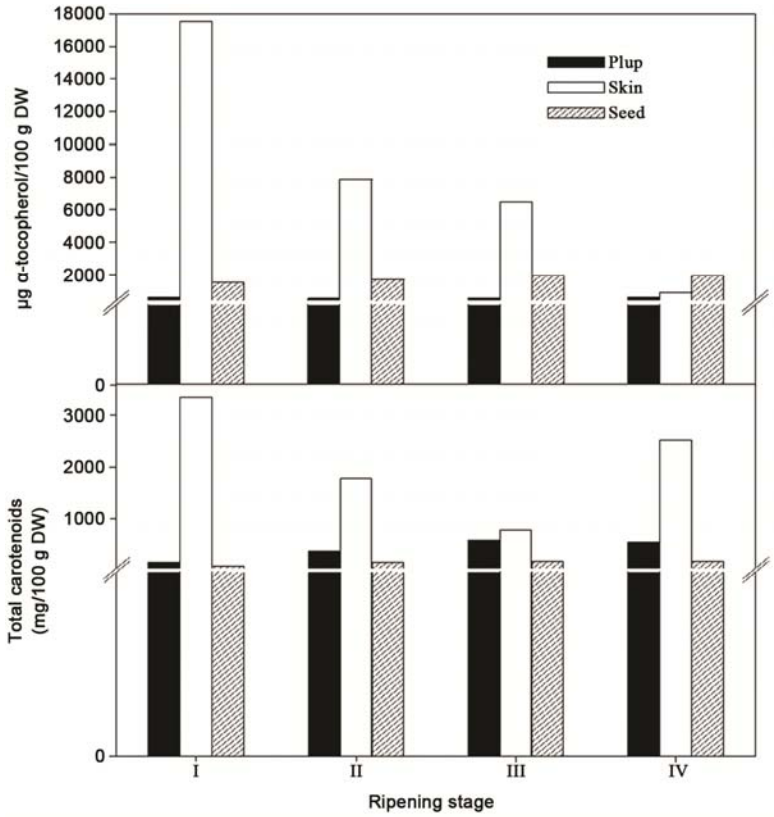

Fig. 3 — α-tocopherol and total carotenoids content of papaya pulp, skin and seeds in different ripening stage. The extraction efficiency based on the average recovery of the internal standard was 95 % for echinenone.

α-tocopherol content during RS I and this one is decreasing during ripening, while in pulp and seeds this parameter does not seem to be affected by the RS. It has been reported that tocopherols are important antioxidants playing a role as protectors of the cell membrane against lipid oxidation by free radicals. Then, the decrease of this compound during ripening in papaya skin could be attributed to a decrease in ascorbic acid, which regenerated the α-tocopherol increasing its capacity to protect the membrane lipids[30]. On the other hand, during fruit and vegetable ripening has been reported an enhancement in the carotenoids biosynthesis. According to our results (Fig. 3), total carotenoids content in papaya pulp increased during ripening, which is in agreement with the pulp colour changes observed (Table 1**),** while papaya skin showed an opposite trend from RS I-III. According to Barreto *et al.*[31], increase in respiration rate and ethylene production during ripening of papaya increased carotenoids content in papaya pulp. The main carotenoids identified in papaya pulp, skin and seeds were β-cryptoxanthin, α-carotene, β-carotene and lycopene (Fig. 4). In skin, in RS III-IV lutein and zeaxanthin were identified, as well as in the seed,

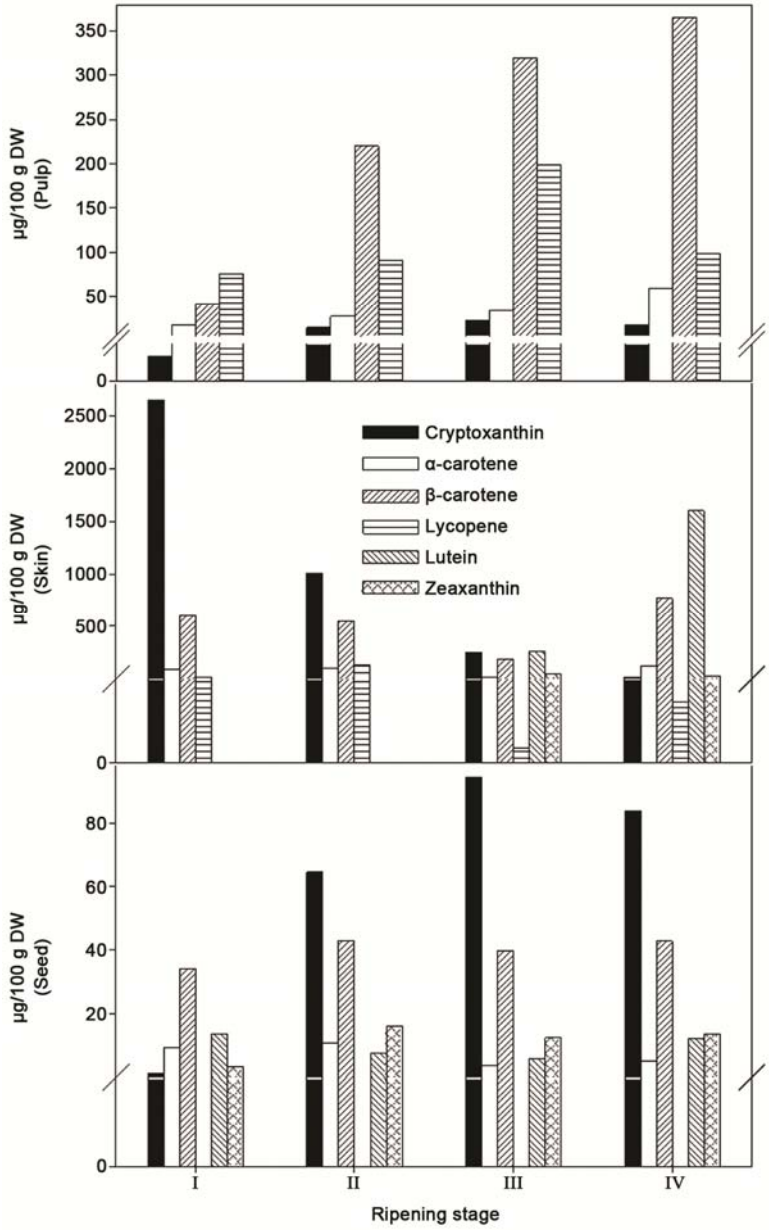

Fig. 4 — Carotenoids content of papaya pulp, skin and seeds in different ripening stage. The extraction efficiency based on the average recovery of the internal standard was 95 % for echinenone.

which presented these last compounds in all RS. In a pulp, Sancho *et al*.[6] and Rivera-Pastrana *et al*.[19] found β-cryptoxanthin, β-carotene and lycopene as main carotenoids in the same papaya cultivar. Colour changes in papaya pulp could be attributed to the high content of β-carotene and lycopene during ripening. Ornelas-Paz *et al*.[18] reported that the best correlation between carotenoids content with a* and Hue values associated with all-trans-β-carotene in mango 'Ataulfo' pulp. However, in papaya, the main carotenoid compound involved with the red colour of the fruit is lycopene. Regarding carotenoids in skin and seeds there is a lack of information, but, it seems that carotenoids are usually more concentrated in peel than in pulp of fruits and fruit vegetables. Lipophilic compounds are important because in the cell membrane prevent oxidation, also, carotenoids have been related to the prevention of a certain type of cancer and cardiovascular disease as Sancho *et al*.[6] mentioned. According to our results, papaya skin resulted to be a good source of α-tocopherol and carotenoids, especially during RS I and RS IV. Taking into account these results, it is convenient to use this byproduct as a source of lipophilic compounds and apply them in the development of functional foods and pharmaceutical ingredients.

Conclusion

Bioactive compounds in papaya pulp, skin and seeds in different RS were determined. Skin and seeds represent a good source of TPC and TFC, while skin is a good source of lipophilic compounds, suggesting its application as antimicrobial and antioxidant ingredients. However, the food industry should take into account the RS of the papaya tissues in regard to the amount of the bioactive compound of interest. Studies about the functional properties of papaya skin and seeds are needed.

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