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# Effect of Maturity Stage of Papaya Maradol on Physiological and Biochemical Parameters

[1,2]Laura E. Gayosso-García Sancho, [3]Elhadi M. Yahia,
[1]Miguel Angel Martínez-Téllez, and [1]Gustavo Adolfo González-Aguilar
[1]Coordination of Food Technology of Plant Origin,
Research Center for Food and Development, AC Km 0.6,
Road to Victory, AP 1735, 83000, Hermosillo, Sonora, Mexico
[2]Graduate Center of the State of Sonora, Federal Labor Law S/N,
CP 83000, Hermosillo, Sonora, Mexico
[3]Faculty of Natural Sciences, Universidad Autónoma de Queretaro,
Avenue of Science S/N, 76230, Juriquilla, Queretaro, Qro., Mexico

**Abstract: Problem statement:** Nowadays, the worldwide increase in diseases has motivated consumers to increase the intake of fruits and vegetables, in response to various research reports indicating that fruits and vegetables can help prevent certain types of illnesses, due to their potentially high antioxidant properties. We evaluated the effect of the stage of ripeness of papaya fruit (*Carica papaya* L.) on the contents of bioactive components and their relation with antioxidant capacity. **Approach:** Whole papaya fruit were selected based on their visual ripeness, classifying them in four stages of ripeness (R1, R2, R3 and R4). Physiological and physical-chemical analysis performed included respiration, production of ethylene, firmness, pH, titratable acidity and total soluble solids, color (L*, a*, b*, °Hue, C); Polygalacturonase (PG) and Pectin Methyl Esterase (PME) activity, total phenolic content and antioxidant capacity (measured using DPPH, TEAC and ORAC assays). **Results:** The antioxidant capacity decreased approximately 27% in the RS4 when using DPPH and TEAC and increased when using ORAC (60.9%). PG activity increased from 8.14 (in RS1)-22.48 U $gFW^{-1}$ (in RS4) as the stage of ripeness of papaya fruit increased. PME was affected in a similar manner with an activity of 0.5562 U $gFW^{-1}$, at the end of the ripening storage. A high correlation between PG activity and softening of ripen papayas was observed. **Conclusion/Recommendations:** It was observed that papaya fruit experienced changes in firmness, which is correlated with activity from two of the main enzymes: PG and PME and with the increase of respiration and production of ethylene. The various stages of ripeness showed very good antioxidant capacity, being higher in RS1, which is correlated with the higher content of phenolic contents found in this ripening stage.

**Key words:** *Carica papaya*, postharvest, antioxidants, phenols, antioxidant capacity

## INTRODUCTION

In the last years, several experimental, clinical and epidemiologist studies have demonstrated that fruits and vegetables contain bioactive compounds with antioxidant and antimicrobial capacity, from different chemical classes such as phenolic compounds, carotenoids, vitamins Gonzalez-Aguilar *et al*. (2008). These were shown to help prevent cardiovascular diseases (Hu, 2003), atherosclerosis, decrease the risk of some types of cancers, among other health benefits (Yahia, 2009).

Papaya is one of the tropical fruits with important antioxidant properties and is also in great demand in international markets. In 2008, Mexico produced about 800,000 tons, of which, the Mexican states of Veracruz and Chiapas contributed with approximately 50% (SAGARPA, 2008). Antioxidant capacity of fruits and vegetables could be affected by a variety of factors, such as: cultivar, agronomic conditions, post-harvest

**Corresponding Author:** Gustavo Adolfo González-Aguilar, Coordination of Food Technology of Plant Origin, Research Center for Food and Development, AC Km 0.6, Road to Victory, AP 1735, 83000, Hermosillo, Sonora, Mexico Fax: +52(662) 280-04-22

manipulation and stage of ripeness (Kevers *et al*., 2007).

Information available on changes of individual phenols and carotenoids in papaya during ripening is limited; therefore methods for the determination of the Antioxidant Capacity (AOC) and for the evaluation of the evolution of papaya during ripening are needed. One of the most commonly used AOC techniques are DPPH (2, 2-diphenyl-1-picrylhydrazyl), TEAC (2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and Oxygen Radical Absorbing Capacity (ORAC) (Corral-Aguayo *et al*., 2008). The first two techniques develop discoloring reactions, which are proportional to the antioxidant capacity in the sample to reduce the radical and are measured using spectrophotometry. With ORAC, a fluorescent protein called fluorescein is used as an oxidable substrate and 2, 2'-azobis (2-amidinopropane) (AAPH) is used as a generator of peroxil radicals and AOC is quantified using a fluorometer. The AOC obtained by these methods are in function of the type and mixture of antioxidants. The objective of this work was to evaluate the effects of maturity stage on physiological and biochemical changes of "Maradol" papaya fruit associated with overall quality.

## MATERIALS AND METHODS

**Plant materials:** Fresh papaya fruit (1-1.5 Kg) (*Carica papaya* L. cv. Maradol) was obtained from a commercial fruit distributor in Hermosillo, Sonora, Mexico and transported to the Fresh-cut Laboratory of the Centro de Investigación en Alimentación y Desarrollo, AC (CIAD). Fruit were selected according to their size, color and external ripeness. Afterwards fruit were sanitized with chlorinated water (200 ppm) for 3 min and were left to dry at room temperature for about 1 h. Fruit were selected subjectively according to surface color and divided in 4 groups of 15 fruit each, where four Ripeness Stages (RS) were established: RS1 represents papaya that is yellow 0-25%; RS2>25 and 50%; RS3>50 and 75% and RS4>75 and 100%.

**Physiological and chemical analysis:** Respiration and ethylene production rates were determined using three pieces of papaya fruit which were selected based on their RS. The 3 pieces of papaya were placed in sealed plastic containers for 2 h. Then, using a hypodermic needle, 1 mL from the headspace was extracted and then injected into a Varian Star 3400 CX gas chromatograph, equipped with the following: A Haysep N column of 200 mm in length and internal diameter of 3 mm; 80/100 µm size; a series of two detectors, one with Thermal Conductivity (TCD) for the quantification of $CO_2$ and the other Flame Ionization (FID) for the detection of ethylene and $N_2$ was utilized as a carrier gas. Temperature conditions were 50°C for the column, 70°C for the injector, 170°C for the TCD detector and 205°C for the FID detector. Concentrations of the standards were 5% $O_2$, 5% $CO_2$ and 1 ppm $C_2H_4$. To determine the concentration of each gas, the area under the curve was integrated and was compared with the areas of the known standards.

Papaya tissue firmness was measured by puncture method, using a Chatillon Penetrometer, Model DFM50 with an 8mm diameter flat-head stainless-steel cylindrical probe. Tissue's opposition force against the penetration was registered on 3 points in the equatorial region of the whole piece of fruit and results were reported in Newtons (N).

The pH, Titratable Acidity (TA) and Total Soluble Solids (TSS) determinations were done following the AOAC (1998) method, where 10 g of fruit were homogenized in 50 ml of distilled water; the mixture was filtered using organza fabric and 50 mL of the filtered mixture were taken to quantify pH and TA using a Mettler (Mod DL21) automatic Tritator. TA was expressed as a percentage of malic acid. TSS were measured directly from the filtered residue, using an Abbe digital refractometer and expressed in °Brix.

Skin color was longitudinally determined on four points of each flat side of the fruit, using a Minolta CR-300 colorimeter. The L* value represents the luminosity of the fruit, where O = Black and 100 = White. The a* value ranges from the negative (green) to the positive (red) scale. The b* value could range from negative (blue) to positive (yellow). To know the real color changes of the fruit, a* and b* values were used to calculate the Hue angle (°Hue) and the Chroma (intensity), with the following equation:

$$°Hue = \arctg b^*/a^*$$
$$C = [(a^*)^2 + (b^*)^2]^{1/2}$$

Where:
°Hue = 0 represents a purple red
90° = Yellow
180° = Green-blue
270° = Blue

**Enzyme assays:** PG activity was determined following the method described by Gross (1982), with some modifications. The samples (10 g) were homogenized in an Ultra Turrax®T25 with 20 mL of 1% sodium bisulfite buffer and 6.0 pH. Next, they were filtered and a second wash of the residue was performed with

20 mL of 1% sodium bisulfite, followed by a third wash with 15 mL of 1M NaCl. The extract's pH was adjusted to 6.0 and then the extract was stirred continuously for 3 hours in a Thermolyne Speci-Mix agitator, at 4°C. Afterward, it was filtered and was centrifuged at 9400 g at 4°C for 15 min. Enzyme solution (250 µL) was mixed well with substrate solution (2 mg polygalacturonic acid dissolved in 750 µL of sodium acetate buffer 37.5 µM, pH 4.4) and was incubated in water bath at 30°C for 2 h. Then the extract was centrifuged at 9400 g at 4°C for 15 min and 200 µL of the supernatant was taken and mixed with 1 mL of 0.1 M borate buffer at 9.0 pH with 200 µL cyanoacetamide (1%). Then the mixture was placed in a water bath at 100°C for 10 min and was left to cool down at room temperature. Absorbance was read using an UV-VIS VARIAN CARY 50 BIO spectrophotometer at 276 nm. Various levels of galacturonic acid solution were used to construct the standard curve (0-100 nmoles) for the PG activity assay. PG activity was expressed as Unit mg $FW^{-1}$ and one activity unit was defined as the amount of enzyme that releases 1 nmol of reducing groups per 1 h. The assay was conducted three times for each RS.

To measure PME activity, a fruit sample (10 g) was homogenized with 25 mL of Tris-Cl 0.1 M buffer at pH 8.0, containing 0.3 M NaCl in an Ultra Turrax®T25 and placed in a Thermolyne Speci-Mix agitator at 4°C for 30 min, followed by centrifugation at 9400 g for 25 min at 4°C. The enzymatic extract was stored at -35°C until analysis and PME was determined following the method of Rouse and Atkins (1955), with some modifications. This method consists of the evaluation of the activity of the enzyme through titration, using as a substrate 25 mL of 1% pectin in 0.1N NaCl at 7.5pH, which was adjusted with 0.1N NaOH. The pectin was placed at water bath at 30°C for 10 min and 2 mL of the extract was added. Decrement of pH caused by the carboxylic groups, generated by the PME during the desertification of the pectin solution were kept constant at a 7.5 pH by titrating the solution with 0.049N NaOH for 10 min at room temperature (24°C). Titration was performed with an automatic Mettler DL21 titrator. Results were expressed as a unit of PME activity, which is defined as the amount that the enzyme required to hydrolyze 1 µmol of carboxyl groups, produced in 1 mL of pectin substrate per minute.

**Biochemical evaluations:** Papaya flesh sample (10 g) was homogenized in 20 mL of 80% methanol, using an Ultra Turrax®T25 basic homogenizer (IKA Works, Willmington, NC) at room temperature. The homogenate flesh was sonicated for 30 min in a Bransonic 2210 sonicator (Bransonic Ultrasonic Co., Danbury, CT) and later was centrifuged at 9400 g for 15 minutes at 4°C. The supernatant was collected and the precipitate was extracted again with 10 mL of 80% methanol, under the conditions previously described. The two supernatants were mixed, filtered using Whatman filter paper No.1 and evaporated in a rotary evaporator at 30°C. The concentrate was diluted with 6 mL of 80% methanol and stored at -35°C to be used in the determination of total phenols, DPPH, TEAC and ORAC. The extraction process was performed in triplicate per each RS.

Total phenols were determined according to Singleton and Rossi (1965), with some modifications. Sample of 50 µL were taken from a 2:8 dilution with 80% methanol) and 3 mL of HPLC-grade water and 250 µL of Folin-Ciocalteu 1N (1:1) reactive were added. After 5 min 750 µL of 20% $Na_2CO_3$ was added, followed by 950 µL of HPLC-grade water; shaken in a vortex and kept in the dark for 30 minutes. Absorbance was read using an UV-VIS VARIAN CARY 50 BIO spectrophotometer, at a wavelength of 765 nm. Results were expressed in mg of Gallic Acid Equivalents (GAE)/100 g of Fresh Weight (FW). Analyses were performed in triplicate per each RS.

DPPH was determined according to the Brand-Williams *et al.* (1995) technique, with some modifications. The stock solution was prepared by mixing 2.5 mg of DPPH radical with 100 mL of pure methanol. The solution was adjusted at an absorbance of 0.7±0.02 at 515 nm. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic) was used as a standard and 80% methanol was used as a blank, 3.9 mL of DPPH radical were placed in a test tube and 100 µL of the extract (2:8 dilution) were added. The mixture was shaken in a vortex and kept 30 min in the dark. Absorbance was then read in an UV-VIS VARIAN CARY 50 BIO spectrophotometer, at a wavelength of 515 nm. Results were expressed in $EC_{50}$ (concentration of antioxidant required to reduce the absorbance of the radical by 50%) in gFW $mL^{-1}$. Analyses were performed in triplicate per each RS.

TEAC value was determined according to Miller *et al*. (1996) and Re *et al*. (1998). $ABTS^{\bullet+}$ cation was generated through the interaction of 19.2 mg of ABTS (2'2-azino-bis(3-ethylbenzotriazoline-6-sulfonic acid)), dissolved in 5 mL of HPLC-grade water and 88 µL of potassium persulfate ($K_2S_2O_8$) (0.0378 g $mL^{-1}$). It was incubated in the dark at room temperature for 16 h; then 1 mL of ABTS activated radical was taken and 88 mL of ethanol was added. The radical was adjusted at an absorbance of 0.7±0.02 at 734 nm. The reaction was initiated adding 2970 µL of $ABTS^{\bullet+}$ and

30 µL of the extract or Trolox standard solution in methanol and. absorbance was monitored at 734 nm at 1 and 6 min. The percentage of inhibition was calculated and results were expressed as µmol of ET/100 gFW.

ORAC value was determined according to Robles-Sanchez *et al*. (2009). AAPH was used as peroxyl radical generator, fluorescein as fluorescent probe and Trolox as standard. The reaction mixture contained 100 µL of extracts, 1.65 mL of 75 mM phosphate buffer (pH 7), 150 µL of 0.8 M AAPH, 100 µL of 0.106 µM fluoresce in and phosphate buffer was used as a blank. Samples, phosphate buffer and fluoresce in were pre-incubated at 37°C for 15 min. AAPH was added to start the reaction and every 5 min the fluorescence was measured and recorded until the fluorescence of the last reading declined to less than 5%, respect to initial. The excitation and emission wavelength was set at 484 and 515 nm, respectively and each extract measurement was repeated 3 times. The values were calculated by using a regression equation between the Trolox concentration and the net area under the fluoresce in decay curve and were expressed as Trolox equivalents (µmol TE) per 100 gFW. The experiment was repeated at least 3 times during the 2009 season.

**Statistical analysis:** All determinations were conducted at least three times. Results were analyzed by multiple comparisons through A Variance Of Analysis (ANOVA) and the statistical significance through the Duncan's test. Differences in $p \leq 0.05$ were considered to be significant. The program Number Cruncher Statistical System version 6.0 software (NCSS, LLC) was used.

## RESULTS

**Respiration rate ($CO_2$ production) and ethylene production:** As expected, the lowest respiration rate was observed in green fruit (RS1) followed by RS2, 3 and 4 (Table 1). These fruit had a respiration rate two-fold higher (15.4 mL $CO_2$/kg.h) than fruit from RS1 (7.8 mL $CO_2$/kg.h). The respiration rate coincided with the ethylene production which increased with the RS of fruit. The highest production of ethylene was observed in RS3 fruit (0.91 µL $C_2H_4$/kg.h), followed by RS4 (0.75 µL$C_2H_4$/kg.h) (Table 1).

**Firmness:** Firmness of papaya flesh tended to diminish with maturity stage and initial values of fruit (RS1), decreasing from 3.9-1.4 N (RS4) (Table 1). However, it is important to point out that although fruit in RS4 presented less firmness, its physical appearance and response to manual pressure was good according to the subjective evaluation. The last fruit was considered the most attractive and acceptable for the consumers.

Table 1: Production of $CO_2$ and $C_2H_4$ and changes in firmness in papaya (*Carica papaya*, L. cv. Maradol) in four stages of ripeness. Mean values in each column followed by a different letter at each ripeness stage are significantly different ($p<0.05$)

| Ripeness stage | $CO_2$ (mL $CO_2$/kg.h) | $C_2H_4$ (µL C2H4/kg.h) | Firmness (N) |
|---|---|---|---|
| 1 (0-25% yellow) | 7.94a | 0.1988[a] | 6.5a |
| 2 (>25 and 50% yellow) | 11.51b | 0.5090b | 3.9b |
| 3 (>50 and 75% yellow) | 14.80c | 0.9108c | 1.9c |
| 4 (>75 and 100% yellow) | 15.41c | 0.7553c | 1.4c |

**pH, TA and TSS:** Maturity stage of papaya did not show a significant effect on pH values, which represents the presence of acidic groups, including organic acids, phenols and amino acids (Table 2). It was observed that in RS1, pH was 6.1 and increased to 6.4 in RS4 (Table 2). TSS varied from 5.4 in RS1-9.4-9.6 for RS3 and RS4, respectively. TA values did not show significant changes in the different maturity stages of papaya fruit.

**Color:** Hue angle (°Hue) represents changes in color of fruit, which ranges from 0 = Red, 90 = Yellow, 180 = Green-blue and 270° = Blue. °Hue value tended to change according to the RS, showing a minimum difference between the RS3 and 4 (Table 2); however, in general, there was a decrease in color (from 124-85). Chroma (C) levels describe the degree of saturation or intensity of color. Results obtained shows that papaya fruit increased their color intensity to similar levels in RS3 y 4, with values ranging from 29-61. These fruits were in good conditions, without apparent mechanical damages in their surface.

**Enzyme assays:** The highest PG activity was observed in fruit RS4 (22.48 U $gFW^{-1}$) and the lowest activity was in the RS1 (8.14 U $gFW^{-1}$) (Fig. 1A). This enzyme is commonly related with fruit softening and increases at the beginning of pectin's desesterification in ripened fruit. PME removes metoxyl groups from small ramifications of pectin or from partially esterified homogalacturonanes changing pectin's solubility, making it more sensitive to the attacks of other enzymes. PME activity usually initiates before that of PG. Figure 1B shows PME's activity in papaya flesh at various RS. PME activity increased gradually at RS1, RS2 and RS3; but decreased to 0.56 U $gFW^{-1}$ in fruit of RS4.

**Biochemical evaluations:** Total phenolic contents of papaya fruit decreased with fruit ripening, with the highest values in RS1 (471.97 and 1.91 mgEAG/100 gFW) and the lowest in RS4 (358.67 and 0.88 mgEAG/100 gFW) in skin and flesh, respectively (Fig. 2). Commonly, phenols presented in higher concentrations in fruit skin than in flesh.

Table 2: Changes in pH, AT, SST and color parameters (L*, a*, b*, °Hue and C) in papaya (*Carica papaya*, L. cv. Maradol) in four stages of ripeness. Mean values in each column followed by a different letter at each ripeness stage are significantly different ($p<0.05$)

| Ripeness stage | pH | AT | SST (°Brix) | L* | a* | b* | °Hue | C |
|---|---|---|---|---|---|---|---|---|
| 1 (0-25% yellow) | 6.141a | 0.074a | 5.4a | 40.01a | -16.20a | 24.86a | 124.69a | 29.68a |
| 2 (>25 and 50% yellow) | 6.207a | 0.062b | 8.7b | 53.30a | -10.98a | 43.98a | 105.00b | 48.08b |
| 3 (>50 and 75% yellow) | 6.302a | 0.056b | 9.4b | 60.73b | 2.74b | 62.79b | 87.50c | 60.50c |
| 4 (>75 and 100% yellow) | 6.401a | 0.048b | 9.6b | 63.47b | 4.86b | 66.40b | 85.81c | 61.66c |

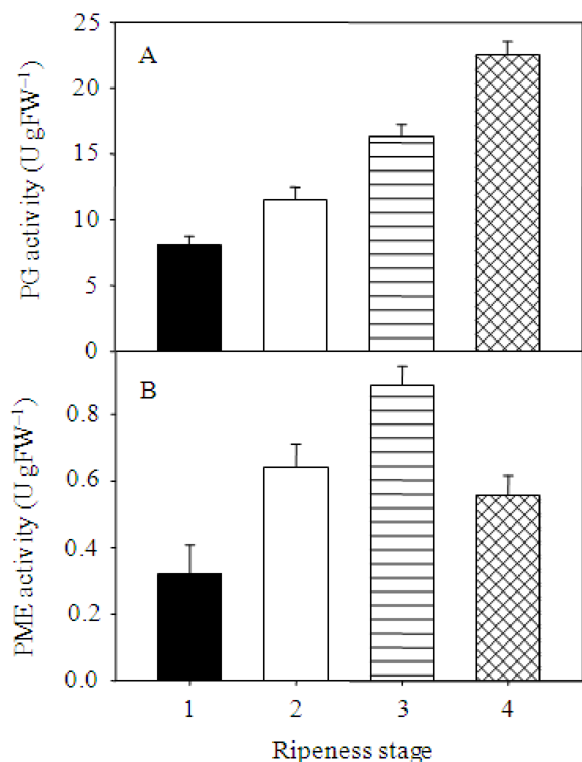


Fig. 1: Activities of polygalacturonase (A) and pectinmethylesterase (B) in papaya (*Carica papaya*, L. cv. Maradol) in four stages of ripeness. Data shows are means of at least three determinations and error bars indicate the standard deviation, expressed as U g $FW^{-1}$

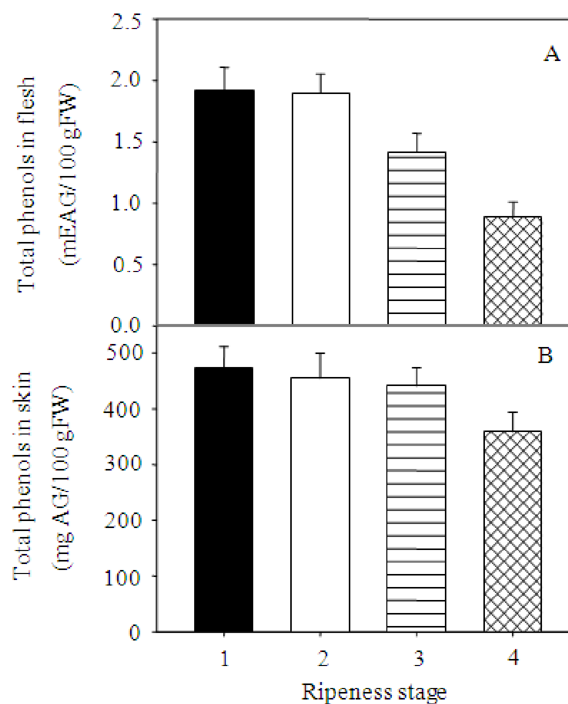


Fig. 2: Totals of phenol in papaya fresh (A) and skin (B) (*Carica papaya*, L. cv. Maradol) in four stages of ripeness. Data shows are means of at least three determinations and error bars indicate the standard deviation, expressed as mEAG/100 gFW

$EC_{50}$ expresses the amount of antioxidants required to reduce by 50% the initial concentration of DPPH radical. Results obtained in the measurement of the AOC of papaya, expressed as $EC_{50,}$ show that RS1 presented the highest antioxidant capacity (0.116 gFW $mL^{-1}$) in skin, while flesh registered 0.313 gFW $mL^{-1}$. The lowest AOC was recorded in RS4, with 0.1378 and 0.616 gFW $mL^{-1}$ for skin and flesh, respectively (Fig. 3A).

The greatest AOC in papaya fruit was recorded at RS1 ripeness, in both the skin and flesh (593.77 and 160 μMET/100 gFW), while the lowest value was at RS4 (547.88 and 116.02 μMET/100 gFW) (Fig. 3B). The antioxidant capacity assessed by ORAC increased as the fruit matured, showing that RS1 had 1065 μmTE/100 gFW, while RS4 had 1714 μmTE/100 gFW (Fig. 3C).

## DISCUSSION

Respiration rate of "Maradol" papaya fruit was similar to that reported in papaya cv. Solo (Paull *et al.*, 1997) with values ranging between 15-35 mL $CO_2$/kg.h at 20°C (Lam, 1990). Several studies on tropical fruit like mango and papaya observed that the higher the level of ripeness and/or storage temperature, the higher the respiration rate (Rivera-Lopez *et al.*, 2005).

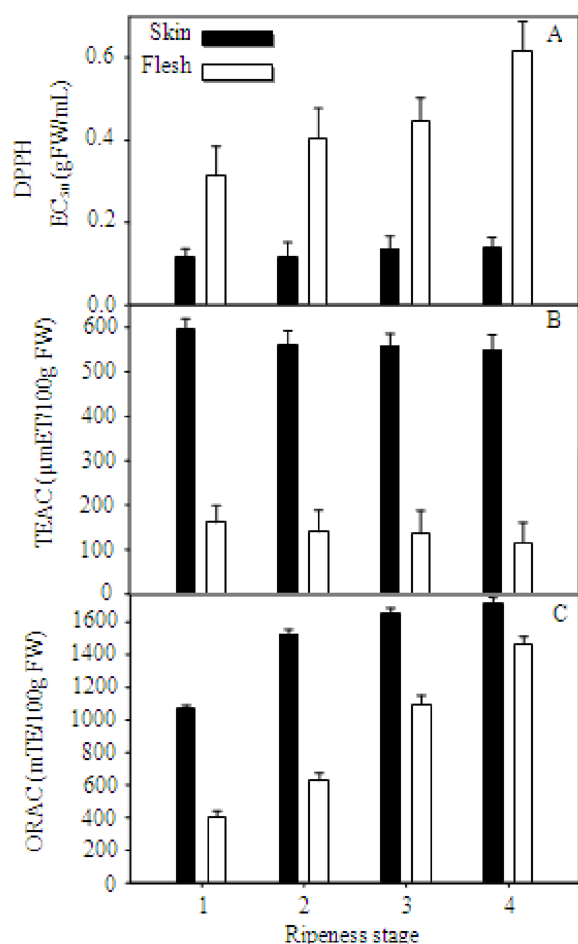


Fig. 3: Antioxidant capacity in papaya fresh and skin (*Carica papaya*, L. cv. Maradol) in four stages of ripeness through DPPH (A), TEAC (B) and ORAC (C). Data shows are means of at least three determinations and error bars indicate the standard deviation

The beginning of ripeness is usually accompanied by an increase of fruit respiration. Climacteric fruits such as papaya are characterized by an increment in their respiration and ethylene biosynthesis patterns during the ripeness process (Lelievre *et al*., 1997). In general, a series of events and reactions occur during the ripeness process, where molecules with high molecular weight (such as starch) are degraded, resulting in molecules with low molecular weight (such as sugars and organic acids, among others). Nevertheless, it is important to point out, that if fruit storage conditions are not appropriate, metabolism accelerates and could exhaust its energetic reserves, resulting in the loss of nutritional value of the fruit (Gonzalez-Aguilar *et al*., 2009).

Ethylene plays an important role in maturation and ripeness. When fruit initiates the ripeness process, small amounts of ethylene (0.1 ppm) can have a profound physiological effect on the fruit, because of its effect on the synthesis of enzymes responsible for physical, chemical and metabolic changes in plant tissue, which influences both firmness and taste of the fruit (Dunkley and Kerith, 1998). Mondal *et al*. (2008) observed that in guava in different RS, the production rate of ethylene increased reaching the climacteric point and then decreased, resulting in firmness loss, due to the activation of enzymes such as PG and PME.

One of the most important factors that affect firmness is the modification in cell walls of fruit and their degradation by pectolitic enzymes. Paull *et al.* (1999) observed an increase in the solubilization of pectin and hemicelluloses with a concomitant firmness loss during ripening of papaya which was enhanced with time and storage temperature. Similar patterns in firmness loss were observed recently in papaya cultivars with respect to our study (Nunes *et al*., 2006). Reduction of firmness observed in papaya correlated with the reduction of °Hue, but the change in color from green to yellow was not always present.

Texture changes have been related to the increase of cell-wall degrading enzymes, which are ethylene dependent. Other studies have reported that firmness loss is caused by the action of PME that remove methyl groups from esterifies galacturonic acids that increase with fruit ripening and enhance the accessibility of PG to its pectic substrate and β-galactosidase activity that increased during the last stages of ripeness (Karakurt and Huber, 2003). Lazan *et al*. (1989) and Chisari *et al*. (2009) observed that the loss of firmness appears first in the internal and later in the external mesocarp, attributing this pattern to the lack of synchronism in the degradation of pectin and hemicelluloses, suggesting that solubilization and depolymerization are two independent events in which PG is responsible for the solubilization of pectins, but not causal of fruit softening.

PG presented the greatest activity in papaya fruit flesh with a RS4 (22.48 U g $FW^{-1}$) and the lowest activity at a RS1 (8.14 U g $FW^{-1}$) (Fig. 1A). It has been reported that PG activity increases at the beginning of pectin's desesterification in ripe fruit and its activity has been correlated with softening of other fruit during ripening (Lohani *et al*., 2004).

PME removes metoxyl groups from small ramifications of pectin or from partially esterified homogalacturonanes. When separating methyl esters, PME not only provides a substrate for PG's action, but also modifies the pH from cell walls, promoting the

action from other enzymes (Chisari *et al*., 2009). Figure 1B shows PME's activity in papaya flesh at various RS, where this activity increased gradually at RS1, RS2 and RS3; but decreased to 0.56 U $gFW^{-1}$ at RS4. A similar behavior has been observed in melons, grapefruits, peaches, kiwis, apples and papaya, where PME's activity *in vitro* decreased during ripening (Chisari *et al*., 2009).

Organic acids provide most of the hydrogen ion sand normally decrease with ripening, producing an increase in pH. Studies performed by Sanudo Barajas *et al.* (2008) obtained a 5.3 pH in green "Maradol" papaya, which shows that pH tends to change, depending on the variety and the degree of ripeness of the fruit. The pH, TA and TSS results obtained in our study were similar to those obtained by others (Corral-Aguayo *et al*., 2008) in whole papaya, TSS tended to increase, while TA tended to decrease with fruit maturation. Storing fruit at low or high temperatures can affect negatively the TSS, as a result of acceleration of ripening (Nunes *et al*., 2006).

The highest color changes are observed when fruit ripe and, in general, the loss of chlorophyll makes yellow and red tones more evident, where carotenoids and other pigments are responsible for these colors. The L* value represents the luminosity or brightness and it was observed that papaya skin presented significant statistical differences ($p\leq0.05$) between the RS1 and RS4 levels, with a tendency to increase, which corresponds to the change of values from 0 to 100 (from dark to light). Nevertheless, this change was minimum in RS3 and RS4 (Table 2). A similar pattern was observed in tomatoes (Marquez and Cortes, 2007) and apples (Rizzolo *et al*., 2006), where luminosity was higher in ripened fruit. Parameter a* shows important changes in the various RS, ranging from negative (green) to positive (red) values, which indicates the loss of chlorophyll and the biosynthesis of carotenoids in the fruit (Yahia and Ornelas-Paz, 2009). With respect to parameter b*, this was used to measure the changes in the fruit from blue to yellow colors and it is possible to observe that in RS1 and 4 exists a considerable change in color, but from RS3-4 there was no significant difference. The result of the increase on the values of parameters a*, b* and L* was similar to the results obtained in studies performed by Ornelas-Paz *et al.* (2008), where L* is correlated with the carotenoid content in the mesocarp of "Manila" mango.

°Hue value tended to change according to the RS, showing a minimum difference between the RS3 and RS4 (Table 2); however, in general, there was a decrease in color (from 124-85). Ornelas-Paz *et al.* (2008) correlated the reduction of Hue values with the contents of the major carotenoids present in mango. Chroma levels describe the degree of saturation or intensity of color. Results obtained show that papaya fruit increased their color intensity from 29-61 in RS3 and RS4.

Fruits and vegetables contain a great number of essential components, which promote health in humans, because of their beneficial effect against certain diseases, especially several cancer pathologies, acting as antivirals, anti-inflammatories and stimulators of immune response; some of these compounds include phenols (Yahia, 2009). Total phenolic contents of papaya in different RS decreased with fruit ripening, the highest values were recorded in RS1 (471.97 and 1.91 mgEAG/100 gFW) and the lowest in RS4 (358.67 and 0.88 mgEAG/100 gFW) in skin and flesh, respectively (Fig. 2). This pattern was similar to that observed in nectarines, peaches and plums, where phenolic contents were 2-6 times greater in the skin than in the flesh (Gil *et al*., 2002).

Mahattanatawee *et al.* (2006) analyzed different tropical fruits and found that ripened papayas (cv. Red Lady) contained the lowest amount of phenolic compounds, which coincides with our study performed recently with eight horticultural crops (Corral-Aguayo *et al*., 2008). Phenolic compounds can act as antioxidants and their activity is determined according with the chemical structure that posses. The reason of the difference between the results obtained in different tropical fruits might be because this capacity increases according to the number of hydroxyls present in fruit and their concentration has been correlated with the antioxidant ability of different types of fruit (Wang *et al*., 2008).

Corral-Aguayo *et al*. (2008) measured the AOC of "Maradol" papaya, obtaining higher values than those obtained in the present study. It is important to point out that this study used methanol as a solvent, therefore results represent the AOC of hydrophylic compounds (like ascorbic acid and phenolic compounds) as well as of those compounds that are not soluble in water, therefore, perhaps the values were lower because they did not react significantly with DPPH radical. On the other hand, Mahattanatawee *et al*. (2006) observed that in green papaya (cv. Red Lady) AOC is higher than in ripe papaya of the same variety, which is in agreement with our results. Studies performed by Gancel *et al.* (2008) in naranjilla indicated that the AOC measured as ED50 (dilution required to reduce by 50% the initial concentration of DPPH radical) was higher in flesh than in skin, which contrasts with the results that we obtained in papaya, where the highest AOC was observed in the skin. The reason for this could be the

interaction of phenolic compounds, organic acids and sugars, as well as the variety and atmospheric conditions, which results in greater AOC. Li-Chen *et al*. (2006) concluded that the AOC obtained in the skin of red pitayas could be the result of the high content and type of phenols (betalains) present in the fruit, because an increment of hydroxyl groups in their molecular structure is related with the increase of AOC.

It is recommended to use different techniques for the AOC evaluation of fruits and vegetables, because of the nature of the different types of compounds present in them and to obtain more reliable results. $ABTS^{\bullet+}$ or TEAC (Trolox equivalent AOC) methodology is widely used for compounds with hydrophilic or lipophylic nature. Also, the ABTS $^{\bullet+}$ radical has been used to confirm results obtained with DPPH, because both possess similar antioxidant mechanisms. It has been reported that phenolic compounds or ascorbic acids react vigorously with ABTS, while lipophylic compounds make them weaker (Perez-Jimenez *et al*., 2008).

The highest AOC in "Maradol" papaya was recorded at RS1 ripeness, in both the skin and flesh (593.77 and 160 µMET/100 gFW), while the lowest value was at RS4 (547.88 and 116.02 µMET/100 gpf) (Fig. 3B). Lako *et al*. (2007) observed that AOC was higher in papaya cv. *Annona* muricata, compared with other types of fruits, attributing these values to the considerable amount of flavonoids present (9 mg/100/g), although generally the content of these compounds is low in fruits.

The AOC assessed by ORAC increased as the fruit matured, showing that RS1 had 1065 µmTE/100 gFW, while RS4 had 1714 µmTE/100 gFW (Fig. 3C). Similar behavior in "Ataulfo" mangoes showed that antioxidant capacity increased during storage at 5 days (Robles-Sanchez *et al*., 2009), which may reflect the contribution of carotenoids (Yahia and Ornelas-Paz, 2009). However, the study performed by Tabart *et al*. (2009) in different fruit and vegetable juices concluded that used methods (ABTS, DPPH and ORAC) could provide widely different results, because some measure lipophylic, hydrophyilic compounds and others do not consider physiological cell conditions.

## CONCLUSION

"Maradol" papaya experienced changes in firmness, which is correlated with activity from two of the main enzymes: PG and PME and with the increase of respiration and production of ethylene. Antioxidant capacity, measured using DPPH, ABTS and ORAC techniques, was higher in RS1, which is correlated with higher content of phenolic contents.

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