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# Effect of ripeness stage of mango fruit (*Mangifera indica* L., cv. Ataulfo) on physiological parameters and antioxidant activity

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ABSTRACT

Many phenolic compounds influence the organoleptic quality of fruits and provide health benefits to consumers due to their antioxidant capacity. Since 'Ataulfo' mango has the highest phenolic content among other mango cultivars, the aim of this research was to investigate how the ripening stage affects their total phenolic content and antioxidant activity. Quality parameters, phenolic content and the antioxidant potential measured by DPPH and FRAP, of mango fruits of four ripening stages (RS) were determined. RS1, representing mango with yellow surface area of 0–10%; RS2, 11–40%; RS3, 41–70% and RS4, 71–100% yellow color. The quality parameters were significantly different ($P \leq 0.05$) in fruits of different RS, except for firmness and pulp color that were similar in fruits from RS3 and RS4. Mango fruits from RS2 and RS3 accumulated the highest phenol content (174 mg EAG/100 g FW) and antioxidant capacity measured by DPPH (93% inhibition). In general, the antioxidant capacity in fruit from the four stages measured by DPPH and FRAP was similar (8.2 μMET/g). In conclusion, RS influences phenolic and flavonoid contents of 'Ataulfo' mango fruit, which was related with the antioxidant capacity of this fruit.



## 1. Introduction

Several clinical and epidemiological studies have demonstrated that fruits and vegetables contain bioactive compounds with antioxidant and antimicrobial activities (Yahia, 2010). These compounds can be of different chemical classes such as phenolic compounds, carotenoids and vitamins (Gonzalez-Aguilar et al., 2008). Mango (*Mangifera indica* L.) fruit can be considered a good source of dietary antioxidants, such as ascorbic acid, carotenoids, and especially phenolic compounds (Ma et al., 2011), which have demonstrated different health-promoting properties, mainly due to their remarkable antioxidant capacity (Kim et al., 2007). Bioactive compounds prevent cardiovascular diseases (Hu, 2003), atherosclerosis, and decrease the risk of some types of cancers, among other health benefits (Yahia, 2010). Thus, regular consumption of mango could provide significant amounts of bioactive compounds with antioxidant activity.

Mango is a popular and economically important tropical fruit throughout the world, due to its excellent eating quality (bright color, sweet taste and luscious flavor) and nutritional composition (vitamins, minerals, fiber, and phytochemicals) (Kim et al., 2009). Global production reached 39 million tons in 2009, followed by banana, pineapple, papaya and avocado (FAOSTAT, 2009). India is the principal mango producer with 35% of the world's production (13.6 million tons), followed by China, Thailand, Indonesia, Mexico and others (FAOSTAT, 2009). However, Mexico is the leading mango-exporting country (41% of the world market), being 'Ataulfo' mango the most important cultivar exported from Mexico to the United States (SAGARPA, 2008).

Recently, it was reported that 'Ataulfo' mango had the highest phenolic content and antioxidant capacity among several mango varieties (Manthey and Perkins-Veazie, 2009). The antioxidant capacity of fruits and vegetables has been correlated to their total phenolic content and composition (Corral-Aguayo et al., 2008). Different factors are reported that affect this antioxidant capacity, such as cultivar, agronomic conditions, post-harvest manipulation and stage of ripeness (Kevers et al., 2007). Although total phenolic compounds have been determined in mango and other tropical fruits, there is a lack of knowledge about the composition and changes of phenolic compounds during maturation and ripening of these fruits.

Various techniques have been developed and used to evaluate the antioxidant capacity of different fruits, and it is suggested to use a combination of at least two of them to estimate the total

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**Fig. 1.** Four selected ripeness stages (RS) in mango (*Mangifera indica* L., cv. Ataulfo). RS1, representing mango with yellow surface area of 0–10%; RS2, 20–30%; RS3, 70–80% and RS4, 100% yellow color.

antioxidant capacity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) methods are the most commonly used, mainly because of their easy performing, high reproducibility and accuracy (Corral-Aguayo et al., 2008; Ma et al., 2011; Vijaya Kumar Reddy et al., 2010).

The nutritional status of fruit is well correlated with the storage life and resistance against different stresses. The long shelf life of 'Ataulfo' mango compared to other cultivars (Kent, Keitt, Haden and Manila) has been attributed to its high vitamin C content and antioxidant potential (Robles-Sánchez et al., 2009a,b). However, the changes in these parameters during ripening of 'Ataulfo' mango are unknown. Therefore the objective of this work was to evaluate the effect of ripening stage on physiological and quality parameters, phenolic content and composition, and antioxidant capacity of 'Ataulfo' mango fruit.

## 2. Materials and methods

### 2.1. Fruit material

Fresh mango fruit (average weight of 200–300 g) (*M. indica* L., cv. Ataulfo) were harvested from a field in Tepic, Nayarit, Mexico, and transported immediately to the laboratory for evaluation. Fruit were selected according to their size, color and appearance discarding fruit with defects and physiological disorders. Afterwards, fruit were sanitized with chlorinated water (200 ppm sodium hypochlorite) for 3 min and left to dry at room temperature (23–26 °C) for about 1 h. Fruit were subjectively selected according to peel surface color and divided in 4 groups of 16 fruits each. Four ripening stages (RS) were established as: RS1, representing mango with yellow surface area of 0–10%; RS2, 20–30%; RS3, 70–80% and RS4, 100% yellow color (Fig. 1). Four mango fruit were taken and the peel was removed with a sharp knife and cut as quickly as possible to obtain the pulp that was cut into small pieces and frozen at −80 °C. After 24 h, the frozen samples were dehydrated in a freeze dryer Labconco Model 1 (Labconco Corp., USA) at −50 °C/0.055 ambar for 36 h, and stored at room temperature in the dark until analyses. The remaining fruit were used for physiological and chemical analysis.

### 2.2. Physiological and chemical evaluations

Pulp and skin color were longitudinally determined on four points of each flat side of 12 fruit, using a Minolta CR- 300 colorimeter (Konica Minolta Sensing, Inc., USA). 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 while the b* value ranges from negative (blue) to positive (yellow) scale. To know the real color changes of the fruit, a* and b* values were used to calculate the Hue angle (°Hue) value.

Respiration and ethylene production were determined using 4 whole fruit per RS. The mango fruit were placed in sealed plastic containers for 2 h. One milliliter from the headspace was withdrawn using a hypodermic needle, and injected into a Varian Star 3400 CX gas chromatograph (Chromatography system, USA), equipped with a Haysep N column (Chromatography system, USA) of 200 mm in length and internal diameter of 3 mm; 80/100 μm size; with two detectors connected in series; a Thermal Conductivity (TCD) and Flame Ionization (FID) for the quantification of $CO_2$ and ethylene, respectively. $N_2$ was utilized as a carrier gas and the 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 used were 5% $O_2$, 5% $CO_2$ and 1 ppm for $C_2H_4$. To determine the concentration of each gas, the area under the curve was integrated and compared with areas of the known standards.

After measuring respiration of Mango, pulp tissue firmness was measured by the puncture method, using a Chatillon Penetrometer, Model DFM50 (Ametek Inc., USA) with 8 mm 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 with skin removed and results were reported in Newton (N).

The pH and total soluble solids (TSS) contents were evaluated in a 10 g sample of the fruit pulp that were homogenized in 50 mL of distilled water; the mixture was filtered and 50 mL of the filtered mixture were taken to quantify pH, using a Mettler automatic Tritator Model DL21 (Corning Scientific Instruments, USA). TSS was measured directly from the filtered residue, using an Abbe digital refractometer (E-Inginst Electron Corp., USA) and expressed as °Brix.

### 2.3. Phenolic content and antioxidant evaluation

Freeze-dried mango pulp samples (1 g) were homogenized in 10 mL solution of 80% methanol and 2% formic acid, using an Ultra Turrax®T25 basic homogenizer (IKA Works, Willmington, NC) at room temperature. The homogenate was sonicated for 30 min in a Bransonic 2210 sonicator (Bransonic Ultrasonic Co., Danbury, CT) and then centrifuged at 9400 × *g* for 25 min 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. The final methanolic extract was stored at −25 °C to be used in the determination of total phenolic acids and flavonoids, and for the DPPH and FRAP assays. The extraction process was performed in six replicates per each RS.

Total phenolic acids were determined according to Singleton and Rossi (1965), with some modifications. Results were expressed in mg of gallic acid equivalents (GAE)/100 g of fresh weight (FW).

Total flavonoids were determined with 5% $NaNO_2$, 10% $AlCl_3$ and 1 mol $L^{-1}$ NaOH (Kim et al., 2003). The reaction was placed in a microplate and absorbance was read using an Omega spectrophotometer (BMG Labtech Inc., Germany) with a microplate reader unit, at 510 nm using catechin as standard. The results were expressed as mg of catechin equivalents (CE)/100 g of fresh weight (FW).

DPPH was determined according to the method reported by Brand-Williams et al. (1995) 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 1.0 ± 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. Samples of 20 μL of the extract (1:10 dilution) were placed in a microplate and 280 μL of DPPH radical were added. The mixture was kept in the dark for 30 min. The absorbance was read using an Omega spectrophotometer (BMG Labtech Inc., Germany) with a microplate reader device, at a wavelength of 490 nm. The inhibition percent was calculated for each sample, which indicates the capacity of the antioxidants to reduce