



## Effects of organic vs. conventional farming systems on quality and antioxidant metabolism of passion fruit during maturation



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### ABSTRACT

The objective of this study was to evaluate the changes in quality and antioxidant metabolism during ripening of passion fruits from organic and conventional production systems. Postharvest quality, variables of antioxidant metabolism (enzyme and non-enzymes), total antioxidant activity (TAA), lipid peroxidation degree as indicator of oxidative stress and the activity of phenolic-associated enzyme, phenylalanine ammonia lyase (PAL) were evaluated in fruits harvested at developmental stages: breaker/turning yellow (physiologically mature) and completely yellow color (ripe). Results show that organic fruits were smaller, although with higher soluble solids content and a lower acidity. At breaker stage, PAL activity was 24% than higher in organic fruits than in conventional fruits, although, total phenolic content was higher in conventional fruits harvested at both breaker (36.14 mg GAE 100 g<sup>-1</sup>) and ripe (36.47 mg GAE 100 g<sup>-1</sup>) stages. Organic fruit showed a significantly greater lipid peroxidation degree of biological membranes, despite their also higher APX activity and vitamin C contents. Results suggest that organic cropping system induced an oxidative stress in passion fruit, negatively influencing fruit size, although inducing the antioxidant defense mechanism, through increments of vitamin C and antioxidant enzymes activity.

### 1. Introduction

*Passiflora edulis* S. or passion fruit is a tropical species rich in vitamin C, B<sub>1</sub> and B<sub>2</sub> and in pro-vitamin A, β-carotene, as well as in minerals and fibers (Freitas et al., 2011). In Brazil, there are over 150 varieties, although, yellow or sour passion fruit represents over 95% of the Brazilian yield (Sabião et al., 2011) and worldwide, Brazil is the third major passion fruit producer with 43 million tons (Santos et al., 2014). The consumption of fruits, as *P. edulis* and other vegetables has been associated with lower risk of chronic human health problems as cardiovascular diseases, cancer, and metabolic diseases due to their high contents of phytochemicals with antioxidant, anti-inflammatory and anticancer properties (McEvoy et al., 2015; Vieira et al., 2015; Sun et al., 2015).

The antioxidant potential of a produce reflects its ability to deliver bioactive substances that neutralize reactive oxygen species (ROS) and

other free radicals produced by oxidative stress. Thus, is determined by the composition, concentration and the efficacy of the antioxidant phytochemicals as vitamins C and E, β-carotene and polyphenols (Zhang et al., 2015; Huan et al., 2016). According to Deshmukh et al. (2011), the concentration of antioxidant compounds in plants is strongly influenced by genetic factors, developmental stage and environment, as climate and soil conditions. Therefore, cropping systems, as organic or conventional may also influence the composition in phytochemicals of fruits and vegetables (Vinha et al., 2014).

During fruit development, ripening is considered as a functionally modified form of senescence associated with ROS accumulation. Thus, mature fruit with high content of antioxidant compounds and activity of antioxidant enzymes could possibly present a greater shelf life with maintenance of quality attributes for longer periods (Mondal et al., 2009; Kumar, 2014). Thus, antioxidant defense mechanism includes enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate

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peroxidase (APX), and of non-enzymatic compounds as ascorbic acid (AsA) and phenolics (Jin et al., 2011; Oliveira et al., 2013).

Environmental influences on the antioxidant capacity of vegetables have been evaluated as studies demonstrated that organically grown fruits and vegetables present better nutritional properties than conventional ones. A comparative study with tomatoes showed that organically grown tomatoes were richer in human health-promoting nutrients than those produced conventionally, with higher phytochemical content and antioxidant activity (Vinha et al., 2014). Moreover, under controlled conditions with good management practices and well-adapted genotypes, the yield of organic systems is similar to that of conventional systems (Seufert et al., 2012). Thereby, based on this information, this study aimed to evaluate quality and antioxidant metabolism of passion fruits from organic and conventional cropping systems.

## 2. Material and methods

### 2.1. Fruit material

Organic and conventional yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.) were obtained from two neighboring commercial farms located in Tanguá-CE, Brazil, situated at 3°51'12"S and 41°5'10"W, at 850 m above sea level. The organic cultivation system used as biofertilizer: animal manure, legume cocktail, sugar cane bagasse or carnauba leaves (carbon source), castor cake (nitrogen source), and rock power (Gafsa®) (phosphorus source), with final nitrogen:carbon ratio of 18:1. Aqueous extract of neem leaves (*Azadirachta indica* A. Juss.) were used as biopesticide on a weekly basis. In the conventional system, pesticides were applied when needed and inorganic fertilizer was used following usual recommendations at the following rates: 300 kg ha<sup>-1</sup> for nitrogen, 120 kg ha<sup>-1</sup> for P<sub>2</sub>O<sub>5</sub> and 320 kg ha<sup>-1</sup> for K<sub>2</sub>O.

Fruit were harvested and evaluated at two developmental stages: breaker/turning yellow (physiologically mature) and completely yellow colored (ripe). Harvest was manual and fruits were carefully selected to insure uniformity of maturity and size, then washed, peels were discarded, the pulp was homogenized using a domestic centrifuge Walita® and stored at -80 °C, until analysis.

### 2.2. Quality attributes

Weight was measured on a semi-analytical scale (Tecnal®, São Paulo-Brazil) as whole fruits were individually weighed and results expressed in grams (g). Size measurements were made with a pachymeter (ZAAS Precision®, São Paulo-Brazil) and expressed as centimeters (cm). Soluble solid (SS) content was determined by refractometry as described by AOAC (2005) using a digital refractometer with automatic temperature compensation (Atago® model N1, Kirkland-USA) and results expressed in °Brix (concentration of sucrose w/w). The pH was measured using an automatic pHmeter (Labmeter PHS-3B®, São Paulo-Brazil) as recommended by AOAC (2005). Titrable acidity (TA) was evaluated as determined by AOAC (2005) recommendations, using an automatic titrator (Mettler-Toledo® DL12, Columbus-USA) and results were expressed as percent (%) of citric acid.

### 2.3. Components of antioxidant metabolism

#### 2.3.1. Non-enzymatic antioxidants

Total vitamin C was determined by titration with Tillman solution (0.02% 2,6 dichloro-indophenol) as described by Strohecker and Henning (1967). One gram of pulp was diluted to 100 mL of oxalic acid and then, homogenized. An aliquot of 5 mL of this solution was added to 50 mL of distilled water and titrated. Results were expressed as mg 100 g<sup>-1</sup> FW.

Total phenolic content was measured by a colorimetric assay using

Folin-Ciocateu reagent as described by Obanda et al. (1997). Before the assay, samples were subjected to extraction in 50% methanol and 70% acetone as described by Larrauri et al. (1997). For the colorimetric assay, 1 mL of Folin-Ciocateu (1:3) reagent, 2 mL Na<sub>2</sub>CO<sub>3</sub> at 20% and 2.850 mL of distilled water were added to 150 µL and 1000 µL of extract for physiologically mature and ripe respectively. After incubation in the dark for 30 min, absorbance was measured at 700 nm and gallic acid was used as the standard, thus results were expressed as gallic acid equivalents (GAE) mg 100 g<sup>-1</sup> FW (fresh weight).

Total anthocyanins and yellow flavonoids were extracted and determined as described by Francis (1982). One gram of pulp was extracted with a 95% ethanol-1.5 M HCl (85:15) solution, vortexed for 2 min and then, brought to 50 mL with the extracting solution. Protected from the light, the mixture was refrigerated at 4 °C for 12 h, then filtered on Whatman N°1 paper and the filtrate was gathered. The absorbance of the filtrate was measured at 535 nm and at 374 nm for total anthocyanins and for yellow flavonoid, respectively. Contents were calculated using absorption coefficients of 98.2 mol cm<sup>-1</sup> for total anthocyanins and 76.6 mol cm<sup>-1</sup> for yellow flavonoids, respectively and results were expressed as mg 100 g<sup>-1</sup> FW.

#### 2.3.2. Total antioxidant activity

The total antioxidant activity (TAA) was determined using ABTS method as described by Rufino et al. (2010). The method consists of measuring the ability of lipophilic and hydrophilic antioxidants to quench a 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS<sup>•+</sup>, Sigma®) radical cation. Before the colorimetric assay, samples were subjected to extraction in 50% methanol and 70% acetone as described by Larrauri et al. (1997). The radical solution was formed using 7 mM ABTS<sup>•+</sup> and 140 mM potassium persulfate, incubated and protected from light for 16 h. Absorbance at 734 nm was measured (approx. to 0.700 ± 0.05) to check for ABTS<sup>•+</sup> formation. Once the radical was formed, the reaction was started by adding 30 µL of extract in 3 mL of radical solution. Absorbance was measured at 734 nm, after 6 min and the decrease in absorption was used to calculate the TAA. A calibration curve was prepared and different Trolox concentrations (standard trolox solutions ranging from 100 to 2000 µM) were also evaluated against the radical. Antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC), µMol Trolox g<sup>-1</sup> FW.

#### 2.3.3. Specific activities of antioxidant enzymes

The extracts for enzymes activity were prepared as described by Yang et al. (2008). Pulp (2 g) was homogenized in an ice-cold extraction buffer (100 mM potassium-phosphate buffer (pH 7.0) + 0.1 mM EDTA). The homogenate was filtered through a muslin cloth and centrifuged at 3000 × g for 40 min. The supernatant fraction was used as a crude extract for antioxidant enzyme activity assays and all the procedures above were performed at 4 °C. The total protein content was determined according to Bradford (1976).

Ascorbate peroxidase (APX, EC 1.11.1.1) activity was assayed according to Nakano and Asada (1981). The reaction mixture (1.5 mL) constituted of 50 mM phosphate buffer (pH 6.0), 0.05 mM EDTA, 0.015 mM ascorbic acid, 0.03 mM H<sub>2</sub>O<sub>2</sub> and 50 µL enzyme extract. The reaction was started by adding ascorbic acid and ascorbate oxidation was measured through absorbance at 290 nm. Enzyme activity was measured using the molar extinction coefficient for ascorbate (2.8 mM cm<sup>-1</sup>) and the results expressed in µmol H<sub>2</sub>O<sub>2</sub> mg<sup>-1</sup> protein min<sup>-1</sup>, considering 1 mol of ascorbate for a reduction of 1 mol H<sub>2</sub>O<sub>2</sub>.

Catalase (CAT, EC 1.11.1.6) activity was measured according to Beers Jr. and Sizer (1952). The reaction mixture (1.5 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 M H<sub>2</sub>O<sub>2</sub> and 37.5 µL enzyme extract. The reaction was started by adding the enzyme extract. The decrease in H<sub>2</sub>O<sub>2</sub> was monitored through absorbance at 240 nm, and quantified by its molar extinction coefficient (36 M<sup>-1</sup> cm<sup>-1</sup>). The results were expressed as µmol H<sub>2</sub>O<sub>2</sub> mg<sup>-1</sup> protein

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