

Ripe Ora-pro-nobis (*Pereskia aculeata miller*) fruits express high contents of bioactive compounds and antioxidant capacity

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Abstract— *Pereskia aculeata* Miller is a native cactaceae found from the Northeast to the South of Brazil. This plant is recognized by the high nutritional value of their leaves, which are the most used in cooking and folk medicine. However, studies on the chemical characteristics and antioxidant capacity of the ora-pro-nobis fruits are scarce. Therefore, the aim of this study was to determine the best harvest point of the ora-pro-nobis fruits to optimize the content of bioactive compounds and their antioxidant capacity. The fruits were manually harvested, defined by their peel colors in three maturity stages: unripe (green), intermediate (yellowish green), and ripe (yellow) stages. The following attributes were evaluated: soluble solids (SS), titratable acidity (TA), SS/TA ratio, pH, and content of bioactive compounds, such as total chlorophylls, carotenoids, yellow flavonoids, total phenolic compounds, and antioxidant capacity. There was a decrease in the content of chlorophyll followed by an increase in the of total carotenoids and yellow flavonoids, due to the maturity stage. For the total phenolic compounds, it was not observed significant difference between the stages. The antioxidant capacity was quantified by the ORAC method in lyophilized pulp in the unripe (1.95 mmol 100 g⁻¹ trolox) and ripe (3.85 mmol 100 g⁻¹ trolox) stages. The lowest contents were observed in the intermediate (0.80 mmol 100 g⁻¹ trolox in lyophilized pulp) stage. The maturity stage significantly influenced the bioactive compounds of ora-pro-nobis, which presented higher values of total carotenoids, yellow flavonoids, and antioxidant capacity in ripe fruits.

Index terms: *Pereskia aculeata* Miller; Maturity index; Harvest point; Postharvest; Brazilian native fruits.

Frutos de ora-pro-nóbis (*Pereskia aculeata miller*) maduros expressam maiores conteúdos de compostos bioativos e capacidade antioxidante

Resumo— *Pereskia aculeata* Miller é uma cactaceae nativa encontrada do Nordeste ao Sul Brasileiro. A planta é reconhecida pelo alto valor nutritivo das folhas, parte mais utilizada na culinária e na medicina popular. Entretanto, trabalhos sobre as características químicas e a capacidade antioxidante dos frutos de ora-pro-nóbis são escassos. Diante disso, o objetivo deste estudo foi determinar o melhor ponto de colheita em frutos de ora-pro-nóbis, visando a otimizar a presença de compostos bioativos e a capacidade antioxidante. Os frutos foram colhidos manualmente, e os três estádios de maturação foram definidos pela coloração da casca, sendo: estádio verde (coloração verde), intermediário (coloração verde - amarelada) e maduro (coloração amarela). Os atributos avaliados foram os sólidos solúveis (SS), acidez titulável (AT), relação SS/AT, pH, e os compostos bioativos, como as clorofilas totais, carotenoides totais, flavonoides amarelos, compostos fenólicos totais e capacidade antioxidante. Houve diminuição da clorofila e aumento no teor de carotenoides totais e flavonoides amarelos, em função do avanço no estádio de maturação. Para os compostos fenólicos totais, não houve diferença significativa entre os estádios avaliados. A capacidade antioxidante foi quantificada pelo método ORAC, em polpa liofilizada no estádio verde, (1,95 mmol 100 g⁻¹ trolox) e maduro (3,85 mmol 100 g⁻¹ trolox). Os menores teores foram observados no estádio intermediário (0,80 mmol 100 g⁻¹ trolox em polpa liofilizada). O estádio de maturação influenciou de forma significativa sobre os compostos bioativos de ora-pro-nóbis, que apresentaram valores mais elevados de carotenoides totais, flavonoides amarelos e capacidade antioxidante em frutos maduros.

Termos para Indexação: *Pereskia aculeata* Miller; Índice de maturação; Ponto de colheita; Pós-colheita; Frutos nativos brasileiros.

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Pereskia aculeata Miller is a creeper plant, which belongs to the Cactaceae family, being native of South and Central America, and tropical America which is naturally distributed from the Northeast to the South of Brazil (SHARIF et al., 2013). It is highlighted as a native plant of excellent potential, being a source of bioactive and nutrient substances (BRASIL, 2004). In Brazil, it is known as ora-pro-nobis, and its leaves are the only part traditionally consumed, being used in culinary and folk medicine (SOUZA et al., 2015). Recent studies have reported the fruits from *Pereskia aculeata* Miller (AGOSTINI-COSTA et al., 2012) and the leaves from *Pereskia grandifolia* Haw (SIM et al., 2010) and *Pereskia bleo* (Kunth) DC. (BAKHARI et al., 2010) are good sources of carotenoids with antioxidant activities.

Although the fruits of ora-pro-nobis have been underused, they are commonly recognized as nutraceutical, or functional and medicinal food, with anti-inflammatory and antinociceptive effects (BARBALHO et al. 2016; SILVA JÚNIOR et al., 2010). The ora-pro-nobis fruits have oval, piriform or rounded shape, yellowish-green, yellow-orange or reddish color (BRASIL, 2010). They are pomaceous, cacti type, with pericarp and seeds dipped in a gelatinous mass (ROSA; SOUZA, 2003). According to AGOSTINI-COSTA et al. (2012), the ora-pro-nobis fruits present protein (1%), fibers (0.7-9.4%), lipids (0.7%), carbohydrates (6.3%); vitamin C (2-125 mg 100 g⁻¹); calcium (174-206 mg 100 g⁻¹), niacin (0.9 mg 100 g⁻¹) and phosphorus (26 mg 100 g⁻¹).

Scarce information on the bioactive compounds and antioxidant capacity of ora-pro-nobis fruits is available; less information about the physicochemical characteristics developed during different maturation stages is also available in the literature. Therefore, this study aimed to determine the best harvesting point in ora-pro-nobis fruits to optimize the presence of bioactive compounds and their antioxidant capacity.

The ora-pro-nobis fruits were collected in June 2014, in a private property, municipality of Campina do Monte Alegre (SP; latitude 23°35'31" S and longitude 48°28'38" W). The fruits were manually harvested in three maturity stages, defined by the peel color. The stages were: unripe (green), intermediate (yellowish green), and ripe (yellow). Then the fruits were transported and selected for no defect to obtain uniform batches.

In the different maturity stages, the fruit pulps were homogenized and submitted to the following analyzes, in triplicates: a) soluble solids (SS) content: quantified in the pulp by direct reading in digital refractometer (Atago PR-101, Palette), and the results were expressed in °Brix (AOAC, 2012); b) titratable acidity (TA): according to methodology described by AOAC (2012); the results were expressed as citric acid percentages; c) SS/TA ratio: obtained by the ratio between the SS and TA contents; d) pH: measured in liquefied samples using a potentiometer,

according to the methodology described by the AOAC (2012); e) content of total chlorophylls and carotenoids: determined by the method described by Lichtenthaler (1987); f) content of yellow flavonoids: determined according to the methodology described by Francis (1982); g) total phenolic compounds (WOISKY; SALATINO, 1998); h) antioxidant capacity by sequestration of the DPPH and ABTS free radicals (RUFINO et al., 2007) and ORAC method (MELO et al., 2015).

The completely randomized experimental design was used, with three replicates (10 fruits each); the data were submitted to analysis of variance and the means were compared using the Tukey's test (5%). The variables were processed using the SAS software, v. 9.3 (2010). Correlations between bioactive compounds (content of total chlorophylls, carotenoids, and total phenolic compounds, yellow flavonoids, and antioxidant capacity by the ORAC method) were calculated using the Statsoft (2004) software and coefficients with indices greater than 0.90 were selected.

During fruit ripening, a 91% increase in the SS/TA ratio between the unripe (2.77) and ripe (5.29) stages was observed (Table 1). Thus, the fruits acquired a more attractive taste for consumption along the maturation stages. The increase in the SS/TA ratio occurred due to an intrinsic ripening processes, mainly degradation of organic acids, and maintenance of soluble solids contents (Table 1). The values for the SS/TA ratio observed in ora-pro-nobis fruits were higher than those evaluated in four passion fruit cultivars (3.15-3.99) (CAVALCANTE et al., 2016). For soluble solids, the difference between the unripe and ripe stages reached the value of 0.45 °Brix. The titratable acidity (TA) of ripe fruits was 1.06% citric acid, which corresponds to a 57% decrease relative to green fruits. Decrease in acidity (about 55%) was also observed in pineapple cv. Vitória fruits when harvested in five different maturity stages (OWAGA et al., 2017).

The pH values measured in ora-pro-nobis fruits were 3.27 in the unripe stage and 3.58 in ripe stage (Table 1). The decrease in the pH value relates to TA and consumption of organic acids during ripening, when these acids act as substrates in the respiratory process (SILVA et al., 2005). However, increase in these values may occur in some fruits along storage, as can be observed in yellow passion fruits (2.74-2.80) (ARAÚJO et al., 2017).

Ripening of the ora-pro-nobis fruits was externally characterized by a marked change in color. With degradation of the green color, result from a decrease in chlorophyll content, predominate the yellow color and the carotenoids pigments. It was observed that the chlorophyll content in the ripe stage of fruits decreased almost three times as compared to the initial value (Table 1). On the other hand, the value of the total carotenoid content in the ripe stage (2.14 mg g⁻¹) contrasted compared to the initial content (1.82 mg g⁻¹) (Table 1). This behavior was

validated by the strong negative correlation obtained between the chlorophyll and total carotenoid variables (Table 2). Qin et al. (2015) also observed an increase in total carotenoid contents in two citrus cultivars at the end of fruit ripening.

Carotenoid and flavonoid pigments have antioxidant properties, associated with the reduction in chronic-degenerative diseases (such as cancer and cardiovascular diseases), besides their anti-inflammatory properties (KAULMANN; BOHN, 2014). The ora-pro-nobis fruits showed an increase in the content of yellow flavonoids between the different ripening stages. In ripe fruits, the contents observed in fresh pulp (9.38 mg 100 g⁻¹) (Table 1) were higher than the mean contents (4.70-6.05 mg 100 g⁻¹) described for passion fruit cultivated in the conventional and organic systems (OLIVEIRA et al., 2017). In the unripe stage, the yellow flavonoids content presented a high negative correlation (-0.99) with the total phenolic compounds contents (Table 2).

The total phenolic compounds did not show statistically significant differences between ripening stages (Table 1). The total phenolic compounds content in ora-pro-nobis fruits were higher than those observed in some traditional fruits, such as pineapple (33 mg GAE 100 g⁻¹), papaya (33.4 mg GAE 100 g⁻¹), and mango (41.1 mg GAE 100 g⁻¹) (SEPTEMBRE-MALATERRE et al., 2016). The contents of bioactive compounds in ora-pro-nobis fruits explicit their potential to complement the human diet (BARBALHO et al., 2016). The antioxidant capacity of native cacti fruits in Brazil is of most importance, since the food-health relationship is being explored and highlighted by the scientific community in this scenario (AGOSTINI-COSTA et al., 2012; ARAUJO et al., 2016; BARBALHO et al., 2016; MORZELLE et al., 2015). The antioxidant capacity was determined using the ORAC (peroxyl radical sequestering) and synthetic DPPH and ABTS free-radical sequestration methods. DPPH and ABTS methods were not capable to detect antioxidant capacity at all ripening stages (Table 1). However, the antioxidant capacity was measured (ORAC method) in the unripe (1.95 mmol 100 g⁻¹ trolox), intermediate (0.80 mmol 100 g⁻¹ trolox), and ripe (3.85 mmol 100 g⁻¹ trolox) stages. Ripe tangerine fruits (*Citrus reticulata* Blanco cv. Chachiensis), presented an oxidative capacity of 1.7 mmol 100 g⁻¹ trolox (WANG et al., 2016). In kiwi fruits (*Actinidia deliciosa*, cv. Hayward), contents of 0.62 mmol 100 g⁻¹ trolox were reported (D'EVOLI et al., 2013). Thus, integrates ora-pro-nobis fruits into the human diet can bring health benefits, since they have a high content of bioactive compounds and a significant antioxidant capacity. In green fruits, a high negative correlation between antioxidant capacity (ORAC) and content of total phenolic compounds was observed, indicating that these components have inverse behaviors at this maturity stage.

Given the results, the progress in the maturity stage positively influences the bioactive and antioxidant compounds of ora-pro-nobis fruits. Harvesting of ripe ora-pro-nobis fruits provide higher content of total carotenoids, yellow flavonoids, and antioxidant capacity.

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Table 1. Chemical characteristics, bioactive compounds, and antioxidant capacity during the different maturation stages of *Pereskia aculeata* Miller fruits harvested in the municipality of Campina do Monte Alegre, São Paulo (SP), Brazil.

Variables	Maturation stages			CV (%)
	Unripe	Intermediate	Ripe	
SS (°Brix)	5.20c	5.35b	5.65a	0.76
TA (% citric acid)	1.87a	1.22b	1.06c	2.30
SS/TA	2.77c	4.38b	5.29a	3.14
pH	3.27b	3.25b	3.58a	3.72
Total chlorophylls (mg g ⁻¹)	1.63a	1.11b	0.67c	5.99
Total carotenoids (mg g ⁻¹)	1.82b	1.64b	2.14a	5.69
Yellow flavonoids (mg 100 g ⁻¹)	5.73b	6.53b	9.38a	7.28
Total phenolic compounds ¹ (mg GAE 100 g ⁻¹)	113.42a	124.03a	120.09a	12.88
ORAC antioxidant capacity (mmol trolox 100 g ⁻¹ lyophilized pulp)	1.95b	0.80c	3.85a	4.38
DPPH (μM Trolox equivalent g ⁻¹ FW)	n.d	n.d	n.d	-
ABTS (μM Trolox equivalent g ⁻¹ FW)	n.d	n.d	n.d	-

In the same line, averages followed by the same letter do not differ significantly from each other (Tukey's test; 5% probability). n.d: not detected.

¹GAE: gallic acid equivalent.

Table 2. Coefficient values for Pearson's linear correlation between bioactive compounds during different maturation stages of *Pereskia aculeata* Miller fruits harvested in the municipality of Campina do Monte Alegre, São Paulo (SP), Brazil.

Variables	Maturation stages														
	Unripe				Intermediate				Ripe						
	TC	TCLR	YF	TPC	ORAC	TC	TCLR	YF	TPC	ORAC	TPC	TCLR	YF	TPC	ORAC
TC	1	-1	0,35	-0,50	-0,50	1	0,50	0,86	0,78	-0,5	1	1	-0,5	-0,54	0,5
TCLR	-1	1	-0,29	0,45	0,45	0,5	1	0,87	0,93	-1	1	1	-0,5	-0,54	0,5
YF	0,35	-0,29	1	-0,99	-0,99	0,86	0,9	1	0,99	-0,9	-0,5	-0,5	1	1	0,5
TPC	-0,50	0,45	-0,99	1	1	0,78	0,9	0,99	1	-0,9	-0,54	-0,54	1	1	0,46
ORAC	-0,50	0,45	-0,99	1	1	-0,5	-1	-0,87	-0,93	1	0,5	0,5	0,5	0,46	1

*TC: total carotenoids; TCLR: total chlorophylls; YF: yellow flavonoids; TPC: total phenolic compounds; ORAC: oxygen radical absorbance capacity. Significant correlations at 5% probability level.

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