

Ozone and temperature on 'Roxinho of Kenya' passion fruit bioactive compounds during storage

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ABSTRACT: Several alternative methods for fruit sanitization have been studied to extend the post-harvest life. Here we investigated whether the sanitization with ozone influences the content of some bioactive compounds during 'Roxinho of Kenya' passion fruit post-harvest. The fruit were immersed in ozonated water, stored at room temperature or under refrigeration for 30 days. Total carotenoids, total phenols, total flavonoids, ascorbic acid, and antioxidant activity in fruit pulp were analyzed. Ozone sanitization did not influenced in the total carotenoids content of fruits stored at room temperature or after immersion for 10 min. in ozonated water and stored in a cold chamber. Sanitization had no influence on total phenol or flavonoid content until day 10 of storage at both temperatures. There was no definite tendency of the ozone action in the ascorbic acid levels or antioxidant activity. There was no microbiological contamination during the storage. The ozone combined with room temperature was unable to maintain the passion fruit post-harvest life and quality. The best conservation method to retain the bioactive levels in 'Roxinho of Kenya' passion fruit is the combination of ozone sanitization for 5 min. and stored in cold chamber for 10 days.

Key words: antioxidants; *Passiflora edulis*; phenolic compounds; sanitization; soluble solids; titratable acidity

Ozônio e temperatura no conteúdo de compostos bioativos durante o armazenamento de maracujá 'roxinho do Kênia'

RESUMO: Diversos métodos alternativos para sanitização de frutos têm sido estudados com a finalidade de prolongar a vida pós-colheita. Nós investigamos se a sanitização com ozônio influencia o conteúdo de alguns compostos bioativos durante a pós-colheita de 'Roxinho do Kênia'. Os frutos foram imersos em água ozonizada, armazenados em temperatura ambiente e sob refrigeração por 30 dias. Foram analisados nas polpas os carotenoides totais, flavonóides totais, ácido ascórbico e atividade antioxidante. Sanitização com ozônio não influenciou o conteúdo de carotenoides totais dos frutos sob temperatura ambiente ou após a imersão por 10 min em água ozonizada e armazenados em câmara fria. Não houve influência da sanitização no teor de fenóis totais e flavonoides totais até o 10o dia de armazenamento em ambas temperaturas. Não houve tendência definida da ação do ozônio sobre o nível de ácido ascórbico ou da atividade antioxidante. Não ocorreram contaminações microbiológicas durante o armazenamento. Ozônio e temperatura ambiente não foram eficientes para manter a qualidade e a longevidade dos maracujás. O melhor método de conservação para reter os níveis de compostos bioativos em maracujá 'Roxinho do Kênia' foi a combinação da sanitização com ozônio por 5 min. e armazenado em câmara fria por 10 dias.

Palavras-chave: antioxidantes; *Passiflora edulis*; compostos fenólicos; sanitização; sólidos solúveis; acidez titulável

Introduction

The passion fruit 'Roxinho of Kenya', or gulupa, (*Passiflora edulis* Sim var *edulis*) is native to tropical and subtropical regions of America and is known for its peculiar characteristics, such as an exotic aroma (Jiménez et al., 2011). It has an intense purple peel, with size of 4 to 6 cm, yellow gelatinous pulp, and sweet to sour flavor. Although this fruit is not regularly produced in Brazil for exportation, the climate is similar to main producer countries. In addition, Brazil shows an interesting potential for production of 'Roxinho of Kenya' with high commercial quality and might be another option for exportation market (Ferraz et al., 2014).

Commonly in fruits the synthesis and accumulation of bioactive compounds vary on function of the species, cultivar, variety, handling, climate, ripening stage, and storage conditions (Severo et al., 2010). In addition, fruit quality maintenance after harvest depends on some factors such as storage conditions and ripening stage in the harvest. Storage conditions have a significant influence on fruit respiratory metabolism and microbial activity, directly determining the post-harvest useful life (Rotili et al., 2013). One of the techniques used to extend the post-harvest life is the refrigeration (Bachmann & Earles, 2000). However, other processes to increase the post-harvest life have been studied, including the use of sanitizers. Generally, the chlorinated compounds are between the most used, especially the hypochlorite salts under the form of chlorinated water. This method may show some disadvantages, as the formation of organochlorinated residues, trihalomethanes and haloacetic acids, which are mutagenic, toxic and carcinogenic, thus limiting its use (Rodgers et al., 2004).

Alternative methods to chlorine have been used, such as ozone, in the food and drinks industry (Lima et al., 2014). The gas decomposes quickly, does not leave residues, and has a higher oxidation power, outperforming even the hydrogen peroxide, the hypochlorite and the chlorine (Alothman et al., 2010). Due to its instability and lack of production of by-products harmful, to the human health or to the environment, ozone has been considered as an alternative to chlorine in the food industry for sanitization (Lima et al., 2014). However, the ozone can induce the oxidative stress since it is a highly oxidative compound. In order to scavenge the reactive oxygen species (ROS), the cells form antioxidant compounds, such as polyphenols, carotenoids, vitamins, among others, which are capable of acting as free radicals scavengers. Thus, herein we aimed to analyze several bioactive compounds in 'Roxinho of Kenya' passion fruit sanitized with ozonated water and stored in different temperatures.

Material and Methods

Fruit were harvested in the first year of production in the São Manuel Experimental Farm (latitude 22°44'50''S and longitude 48°34'00''W, altitude 765 m), Botucatu, São Paulo,

Brazil, when they reached their commercial ripening point (peel of intense purple color). The climate of the region is classified as warm and humid with a little hydric deficiency in the months of April, July and August. The rainfall is concentrated from November to April, with annual rain average precipitation of 1,376 mm and average temperature during the hottest month higher than 22 °C (Cunha & Martins, 2009).

To analyses were selected fruit with no signs of mechanic damages or injuries were selected. Fruit were washed in tap water in order to remove larger impurities, separated into three homogeneous groups and submitted to sanitization treatments with ozonated water generated by an ozone generator (Degradatox/OZ Engenharia, Industrias LTD Equipamentos, Porto Alegre, Brazil; generating 1 mg L⁻¹ s⁻¹). Immersion in ozonated water was performed for 5 (O₃-5) and 10 (O₃-10) minutes. Control fruit were washed with tap water. After the sanitization process, fruit were placed in open bottom polystyrene trays and subdivided in two groups according to the storage temperature, thus defining the experimental unity. Fruit were stored in cold chamber (10 ± 2 °C and 90 % RH) and the other part were stored in room temperature (20 ± 2 °C and 71.8% RH) for 30 days. The analyses, in triplicate, were performed at each 5 days, using four fruit per analysis.

Fruit were cut with a stainless steel knife, and the pulp was squeezed-out and filtered through a fine polypropylene sieve. After the pulp separation, the samples were pulverized in liquid nitrogen and stored in freezer at - 80 °C.

We assessed the pH was determined using a digital pHmeter (Quimis®). To quantifying the soluble solids (SS) an electronic refractometer (Schmidt + Haensch) was used, and the results were expressed in °Brix (IAL, 2008). The titratable acidity (TA) was also determined according to the methodology recommended by IAL (2008), using 2 g of sample (pulp) homogenized and diluted in 100 mL of deionized water, followed by titration with buffer solution of 0.1 N NaOH and phenolphthalein turning point as the indicator. The results were expressed in g of citric acid per 100 g⁻¹ of pulp sample.

Total carotenoids were determined according to the method proposed by Sims & Gamon (2002). The pulp was homogenized with 80% Tris-acetone buffer, pH 7.8 and after centrifugation (5 min, 2000 g (Hettich Zentrifugen, Mikro 220R), 4 °C), the supernatant was collected and the reading was performed at 663 nm for chlorophyll A, 647 nm for chlorophyll B, 537 nm for anthocyanin and 470 nm for carotenoids (Pharmacia Biotech, Ultrospec 2000). Results were expressed in mg 100 g⁻¹.

The total phenolic compounds content was determined according to the spectrophotometric method, using Folin-Ciocalteu (Singleton & Rossi, 1965). The samples were weighted and 5 mL of 50% acetone was added. After 20 min in ultrasonic bath (Elma, Transsonic 420), the samples were centrifuged at 5000 g (Hettich Zentrifugen, Mikro 220R), during 10 min. The supernatant was collected and

the precipitate was submitted again to the same process and, finally, the supernatants were combined and the total phenolic compounds analysis was performed. The reading was performed at 725 nm (Pharmacia Biotech, Ultrospec 2000) and the results were expressed in mg gallic acid equivalent 100 g⁻¹.

Flavonoids were quantified according to Popova et al. (2004). The samples were homogenized with 10% methanol-HCl (85:15 v/v) and submitted to ultrasonic bath. After 20 minutes it was added 5% AlCl₃ (p/v), and centrifuged for 20 minutes at 10.000 g (Hettich Zentrifugen, Mikro 220R), at 5 °C. The reading was performed in 425 nm (Pharmacia Biotech, Ultrospec 2000) and the results were expressed in mg rutin equivalent 100 g⁻¹.

The ascorbic acid content was determined by titration: 2 g of pulp were homogenized in 30 mL of 1% oxalic acid and subsequently titrated with 2 % 2,6-dichlorophenol-indophenol (IAL, 2008). The results were expressed in mg 100 g⁻¹.

Antioxidant activity was carried out in ethanol, according to Brand-Williams et al. (1995). Samples were homogenized in absolute ethanol and after 15 min in ultrasonic bath, the samples were centrifuged for 10 min. at 6000 g (Hettich Zentrifugen, Mikro 220R). All samples analysis were conducted in triplicate. Readings were performed at 517 nm (Pharmacia Biotech, Ultrospec 2000), and the results expressed in percentage of antioxidant activity using the following equation:

$$\% \text{ reduced DPPH} = \left(\frac{\text{Absorbance blank} - \text{Absorbance sample}}{\text{Absorbance blank}} \right) \cdot 100$$

Microbiological analysis were made using techniques proposed by Lima et al. (2013), by total plate counts (TPC), yeasts, and molds and coliform.

For the statistical analyses, we used an entirely randomized design in factorial scheme 3 x 7 for the refrigeration and factorial scheme 3 x 6 for room temperature, with two types of sanitization, beyond the control (O₃-5, O₃-10, Control) and shelf life (0, 5, 10, 15, 20, 25 e 30 days), whit four repetitions. All obtained data were submitted to the variance analysis (Test F). All obtained data were submitted to analysis of variance (Test F). The averages between treatments were compared using the Scott-Knott test at 5% probability. The results were expressed as the mean of determinations ± SD. The software Statistica version 7.0 was utilized (Stat Soft. Inc.).

Results and Discussion

Storage at room temperature in combination with ozone sanitization, significantly affected the 'Roxinho of Kenya' passion fruit post-harvest quality. This effect was more significant in fruit sanitized by ozonated water for 10 minutes, which showed a durability of 15 days, in contrast to 25 days verified for the fruit washed only with water. One of the most important factors influencing fruit durability is the

effects of temperature on respiratory metabolism and the consequent gradual reduction of the shelf life of fruits and vegetables (Rotili et al., 2013). In addition to the temperature effect, the ozone produced an oxidating effect, which can cause tissues damage (Monaco et al., 2016). According to Palou et al. (2002), grapes or peaches treated with ozone did not show changes in the patterns that limitate the storage time, such as respiratory rates or ethylene production. Meanwhile, ozonated water was used as an alternative to chlorine sanitizer for mango without causing damage (Monaco et al., 2016). In our study, the ozone sanitization in combination with the room temperature condition may have promoted damage due to the oxidating effect, thus changing the quality and decreasing the shelf life of the 'Roxinho of Kenya' passion fruit.

In the cold chamber, the fruit reached 30 days of shelf-life, regardless of the sanitization method employed. The storage of fruit at low temperatures slows down the metabolism, decreasing the respiratory rate and the enzymatic activity, delaying the senescence, and influencing the aroma, flavor, color, texture and other quality attributes of the stored product (Antunes et al., 2003).

Total plate counts (TPC) and yeast and mold counts were carried out for microbiological analysis at up to 30 days of storage. We found no microbiological contamination detected in the control or ozonated fruits during at any time of storage (data not shown).

There was an increase of pH during the storage time under room temperature, regardless of the sanitizing treatment. This response can be attributed to the utilization of organic acids by fruit, which promotes the decrease in acidity and increase in pH (Pinzón et al., 2007). In opposition, in the refrigerated environment, the fruit showed no pH variations until day 15 (Table 1). With the pH decrease there was an increase of TA in the fruit stored under room temperature. When the fruit were sanitized with ozonated water for 5 or 10 minutes and stored in a cold chamber, the sanitization process did not cause any alterations in the titratable acidity of the fruit. Other studies have also shown that the ozone does not lead to modifications in the acids levels in fruits and vegetables, as described for kiwifruit (Minas et al., 2010). Acidity is one of the criteria that influence the classification of fruits based on their flavor and the accumulation of organic acids levels that occurs during the entire fruit development. Generally, there is a decrease in the acids content after the harvest, due to changes in metabolic processes, such as an increase in the respiration (Ali et al., 2014; Barboni et al., 2010).

Soluble solids decreased in all fruit, regardless of the treatment (Table 1). SS are used as indicators of ripeness and fruit quality and they play an important role in the flavor. In this study, we found that the ozone did not influence the SS content, except at day 30, when fruit under refrigeration and immersed in ozonated water for 10 minutes showed the lowest SS contents. These results are different from what has been found in other fruits treated with ozone, such as kiwi,

Table 1. pH, titrable acidity and soluble solids in 'Roxinho of Kenya' passion fruit sanitized with ozonated water and stored in ambiente temperature and in cold chamber.

Storage (days)	pH			SS			TA		
	Control	O ₃ -5min	O ₃ -10min	Control	O ₃ -5min	O ₃ -10min	Control	O ₃ -5min	O ₃ -10min
Room temperature									
0	3,15 ± 0,07 ^{Ac}	3,19 ± 0,09 ^{Ac}	3,14 ± 0,04 ^{Ac}	15,48 ± 0,61 ^{Ba}	16,45 ± 0,21 ^{Aa}	16,35 ± 0,39 ^{Aa}	3,25 ± 0,19 ^{Aa}	2,80 ± 0,16 ^{Ba}	3,00 ± 0,16 ^{Ba}
5	3,19 ± 0,04 ^{Ac}	3,18 ± 0,04 ^{Ac}	3,23 ± 0,05 ^{Ab}	15,58 ± 0,41 ^{Ba}	16,35 ± 0,06 ^{Aa}	14,98 ± 0,42 ^{Bb}	3,20 ± 0,00 ^{Aa}	2,90 ± 0,12 ^{Ba}	2,75 ± 0,19 ^{Ba}
10	3,46 ± 0,08 ^{Ab}	3,31 ± 0,04 ^{Bb}	3,30 ± 0,06 ^{Bb}	14,35 ± 0,39 ^{Bb}	15,23 ± 0,53 ^{Ab}	14,80 ± 0,22 ^{Ab}	2,25 ± 0,19 ^{Cb}	2,50 ± 0,12 ^{Bb}	2,90 ± 0,20 ^{Aa}
15	3,42 ± 0,04 ^{Bb}	3,42 ± 0,07 ^{Ba}	3,51 ± 0,06 ^{Aa}	14,73 ± 0,33 ^{Cb}	15,85 ± 0,65 ^{Bb}	16,65 ± 0,39 ^{Aa}	2,05 ± 0,10 ^{Ac}	2,15 ± 0,30 ^{Ac}	1,65 ± 0,19 ^{Bb}
20	3,52 ± 0,02 ^{Aa}	3,47 ± 0,02 ^{Aa}	-	15,75 ± 0,56 ^{Bb}	15,48 ± 0,43 ^{Ab}	-	2,00 ± 0,16 ^{Ac}	1,35 ± 0,10 ^{Bd}	-
25	3,57 ± 0,04 ^a	-	-	15,10 ± 0,36 ^a	-	-	2,45 ± 0,19 ^b	-	-
Cold chamber									
0	3,15 ± 0,07 ^{Ab}	3,19 ± 0,09 ^{Ab}	3,14 ± 0,04 ^{Ac}	15,48 ± 0,61 ^{Bb}	16,45 ± 0,21 ^{Aa}	16,35 ± 0,39 ^{Aa}	3,25 ± 0,19 ^{Aa}	2,80 ± 0,16 ^{Bb}	3,00 ± 0,16 ^{Ba}
5	3,17 ± 0,09 ^{Bb}	3,27 ± 0,06 ^{Aa}	3,15 ± 0,06 ^{Bc}	15,73 ± 0,33 ^{Ab}	16,68 ± 0,34 ^{Bc}	16,13 ± 0,51 ^{Aa}	3,00 ± 0,16 ^{Ab}	3,05 ± 0,30 ^{Aa}	3,20 ± 0,00 ^{Aa}
10	3,16 ± 0,04 ^{Ab}	3,19 ± 0,08 ^{Ab}	3,20 ± 0,04 ^{Ac}	16,30 ± 0,24 ^{Aa}	15,93 ± 0,22 ^{Ab}	15,30 ± 0,18 ^{Bb}	3,20 ± 0,23 ^{Aa}	3,35 ± 0,10 ^{Aa}	3,35 ± 0,10 ^{Aa}
15	3,15 ± 0,09 ^{Ab}	3,20 ± 0,07 ^{Ab}	3,22 ± 0,07 ^{Ac}	15,55 ± 0,37 ^{Ab}	15,48 ± 0,49 ^{Ab}	15,75 ± 0,42 ^{Ab}	3,20 ± 0,16 ^{Aa}	3,15 ± 0,10 ^{Aa}	3,35 ± 0,10 ^{Aa}
20	3,05 ± 0,03 ^{Ac}	3,07 ± 0,02 ^{Ac}	3,04 ± 0,04 ^{Ad}	15,85 ± 0,70 ^{Ab}	15,55 ± 0,41 ^{Ab}	15,38 ± 0,10 ^{Ab}	3,25 ± 0,19 ^{Aa}	3,30 ± 0,12 ^{Aa}	3,20 ± 0,16 ^{Aa}
25	3,23 ± 0,03 ^{Aa}	3,26 ± 0,07 ^{Aa}	3,27 ± 0,05 ^{Ab}	15,68 ± 0,40 ^{Ab}	14,95 ± 0,26 ^{Bc}	15,45 ± 0,35 ^{Ab}	3,30 ± 0,12 ^{Aa}	3,20 ± 0,16 ^{Aa}	3,15 ± 0,19 ^{Aa}
30	3,31 ± 0,06 ^{Aa}	3,28 ± 0,07 ^{Aa}	3,37 ± 0,05 ^{Aa}	14,18 ± 0,48 ^{Ac}	14,43 ± 0,29 ^{Ac}	13,00 ± 0,22 ^{Bc}	2,95 ± 0,19 ^{Bb}	3,20 ± 0,23 ^{Aa}	3,30 ± 0,12 ^{Aa}

*Means followed by the same capital letter in the line and lowercase in the column do not differ between themselves by the *Scott-Knott* test at 5% of significance. O₃, ozone sanitization.

where there was no variations in the SS content (Minas et al., 2010). The authors attributed an anti-senescent effect to ozone, which can extend the post-harvest life. However, as these results were not verified in 'Roxinho of Kenya' passion fruit, it would be important to perform complementary studies with other fruits from the genus *Passiflora*.

The use of ozonated water (5 or 10 min.) promoted alterations in the total carotenoids content from 10^o storage day at room temperature (Table 2). When stored in cold chamber, untreated passion fruit showed higher content at day 30, while the fruit sanitized with ozonated water for 10 min. did not show variations during the storage.

The use of ozone, either gaseous or dissolved in water, can cause loss of some antioxidant components, due to its oxidizing effects (Tiwari et al., 2008). However, as the ozone decomposition produces free radicals, mainly hydroxyls (Graham, 1997), it can occur an increase in the antioxidant activity can occur as a defense against ROS. In this study, the exposure of 'Roxinho of Kenya' passion fruit to ozonated water (5 or 10 min.) did not induce significant alterations in the carotenoids content when compared to the control and this effect is more noticeable in fruit stored in room temperature. In tomatoes sanitized with gaseous ozone,

Tzortzakis et al. (2007) describe an increase of carotenoid content (2 to 3 times), 24 hours after exposure; however, the effect did not persist and at six days after the treatment, the carotenoids levels did not show differences from the untreated fruit. In our study, the first analysis was performed only on the fifth day after the sanitization with ozonated water, which may have concealed the immediate effect on carotenoid content. In contrast, this increase may be irrelevant to the consumer because the fruits are not usually consumed immediately after the harvest.

Regardless of the sanitization treatment or storage temperature, there was a decrease in the phenolic compounds content on 10th day (Table 3). Similar results are also found for the total flavonoids content is analyzed. Regardless of the treatments applied, total phenols and flavonoids tend to increase in 'Roxinho of Kenya' passion fruit after 20 days of storage (Table 3). Many studies have demonstrated the effects of ozone on the phenolic compounds content, with contradictory results. Increases in the phenols or flavonoids levels in pineapple and banana treated with ozone were described previously (Allothman et al., 2010). The authors attributed this effect to the ozone antioxidant activity in the cell wall, compared to untreated fruit. Minas et al. (2010)

Table 2. Total carotenoids content of 'Roxinho of Kenya' passion fruit sanitized with ozonated water and stored in two temperatures.

Storage (days)	Total carotenoids (mg 100 g ⁻¹)					
	Room temperature			Cold chamber		
	Control	O ₃ -5min	O ₃ -10min	Control	O ₃ -5min	O ₃ -10min
0	0,39 ± 0,03 ^{Ba}	0,51 ± 0,09 ^{Aa}	0,55 ± 0,14 ^{Aa}	0,39 ± 0,03 ^{Bc}	0,51 ± 0,09 ^{Aa}	0,55 ± 0,14 ^{Aa}
5	0,39 ± 0,07 ^{Aa}	0,46 ± 0,07 ^{Aa}	0,48 ± 0,05 ^{Aa}	0,45 ± 0,03 ^{Ac}	0,44 ± 0,09 ^{Ab}	0,54 ± 0,06 ^{Aa}
10	0,30 ± 0,05 ^{Ab}	0,28 ± 0,05 ^{Ab}	0,38 ± 0,04 ^{Ab}	0,40 ± 0,07 ^{Ac}	0,42 ± 0,10 ^{Ab}	0,40 ± 0,08 ^{Aa}
15	0,31 ± 0,07 ^{Ab}	0,33 ± 0,13 ^{Ab}	0,31 ± 0,06 ^{Ab}	0,35 ± 0,11 ^{Ac}	0,37 ± 0,05 ^{Ab}	0,34 ± 0,06 ^{Aa}
20	0,26 ± 0,05 ^{Ab}	0,29 ± 0,06 ^{Ab}	-	0,31 ± 0,05 ^{Bc}	0,33 ± 0,09 ^{Bb}	0,44 ± 0,03 ^{Aa}
25	0,38 ± 0,02 ^a	-	-	0,63 ± 0,07 ^{Aa}	0,41 ± 0,06 ^{Bb}	0,47 ± 0,10 ^{Ba}
30	-	-	-	0,51 ± 0,04 ^{Ab}	0,52 ± 0,10 ^{Aa}	0,46 ± 0,03 ^{Aa}

*Means followed by the same capital letter in the line and lowercase in the column do not differ between themselves by the *Scott-Knott* test at 5% of significance. O₃, ozone sanitization.

Table 3. Ozonated water and action storage temperature on the total phenol and total flavonoids of 'Roxinho of Kenya' passion fruit.

Storage (days)	Phenols (mg 100 g ⁻¹)			Flavonoids (mg 100 g ⁻¹)		
	Control	O ₃ -5 min	O ₃ -10 min	Control	O ₃ -5 min	O ₃ -10 min
Cold chamber						
0	39,84 ± 0,71 ^{Bd}	41,32 ± 0,84 ^{Ac}	41,36 ± 0,44 ^{Ac}	6,35 ± 0,67 ^{Bd}	10,73 ± 0,64 ^{Ab}	11,01 ± 0,64 ^{Ab}
5	43,15 ± 0,90 ^{Ac}	41,84 ± 0,75 ^{Bc}	43,39 ± 0,85 ^{Ab}	10,14 ± 0,46 ^{Ab}	10,01 ± 0,28 ^{Ac}	8,35 ± 0,99 ^{Bd}
10	39,26 ± 0,34 ^{Bd}	41,63 ± 0,99 ^{Ac}	38,05 ± 0,33 ^{Ce}	7,94 ± 0,71 ^{Ac}	7,61 ± 0,78 ^{Ad}	7,12 ± 0,52 ^{Ae}
15	33,30 ± 0,49 ^{Be}	40,51 ± 0,76 ^{Ad}	31,32 ± 0,77 ^{Cf}	7,37 ± 0,19 ^{Bc}	9,61 ± 0,53 ^{Ac}	9,32 ± 0,49 ^{Ac}
20	44,16 ± 0,49 ^{Ab}	45,16 ± 0,88 ^{Ab}	44,69 ± 0,43 ^{Aa}	10,23 ± 0,82 ^{Ab}	10,71 ± 0,89 ^{Ab}	9,86 ± 0,63 ^{Ac}
25	44,58 ± 0,70 ^{Ab}	44,94 ± 0,73 ^{Ab}	39,75 ± 0,96 ^{Bb}	11,26 ± 0,19 ^{Aa}	11,18 ± 0,45 ^{Ab}	9,75 ± 0,76 ^{Bc}
30	51,34 ± 0,82 ^{Ba}	53,22 ± 0,60 ^{Aa}	44,36 ± 0,52 ^{Ca}	12,06 ± 0,61 ^{Ba}	13,35 ± 0,62 ^{Aa}	12,30 ± 0,64 ^{Ba}
Room temperature						
0	39,73 ± 0,17 ^{Bd}	41,32 ± 0,55 ^{Ac}	41,36 ± 0,44 ^{Ab}	6,39 ± 0,42 ^{Bb}	10,73 ± 0,64 ^{Aa}	10,82 ± 1,19 ^{Ac}
5	41,37 ± 0,95 ^{Ac}	40,61 ± 0,70 ^{Ac}	35,61 ± 0,72 ^{Bd}	11,60 ± 0,61 ^{Ba}	10,12 ± 0,65 ^{Cb}	14,98 ± 0,66 ^{Aa}
10	26,38 ± 0,99 ^{Ce}	32,31 ± 1,37 ^{Bd}	36,80 ± 1,33 ^{Ac}	10,83 ± 0,21 ^{Ba}	11,43 ± 0,76 ^{Ba}	12,70 ± 0,98 ^{Ab}
15	39,62 ± 1,00 ^{Cd}	50,03 ± 1,21 ^{Ab}	42,89 ± 1,23 ^{Ba}	11,41 ± 0,65 ^{Aa}	11,67 ± 0,84 ^{Aa}	11,31 ± 0,42 ^{Ac}
20	63,89 ± 0,72 ^{Aa}	56,68 ± 0,65 ^{Ba}	-	11,15 ± 0,72 ^{Aa}	9,05 ± 0,72 ^{Bc}	-
25	56,28 ± 0,60 ^b	-	-	11,92 ± 0,28 ^a	-	-

* Means followed by the same capital letter in the line and lowercase in the column do not differ between themselves by the Scott-Knott test at 5% of significance. O₃, ozone sanitization.

also reported an increase in the phenolic compounds content in kiwi after ozone treatment. Other authors found no difference in the polyphenols content in fruit treated with ozone, as described for tomato (Tzortzakis et al., 2007).

The polyphenols content in fruits and vegetables is determined by genetic factors, but can change due to oxidative reactions caused by biotic and abiotic factors, including temperature, transpiration, oxygen and post-harvest (Rotili et al., 2013). We expected that the use of ozonated water would affect the total phenols content, mainly flavonoids. In the literature, the use of ozone has shown many effects on the phenolic compound content, including flavonoids. This effect is attributed to the activation of phenylalanine ammonia lyase (PAL), an enzyme that regulates the biosynthesis of phenolic compounds, which can be stimulated by many stress factors (Yeoh et al., 2014). In 'Roxinho of Kenya' passion fruit this effect was not observed, thus the immersion in ozonated water treatments for 5 or 10 minutes were not effective in modifying the PAL activity and, consequently, increasing the flavonoid levels when stored at room temperature or in a cold chamber.

'Roxinho of Kenya' passion fruit stored at room temperature and immersed only in water showed an

ascorbic acid increase at day 5; however, when the fruit were immersed in ozonated water this behavior was not repeated (Table 4). The highest ascorbic acid content in the fruit ozonated for 5 min occurred at day 10. In contrast, in same period, the ascorbic acid levels were lower in fruit ozonized for 10 min. Under refrigeration, both at 10 and 15 days, the fruit immersed in ozonated water showed the highest vitamin C content. Due to variations found in the vitamin C content, both the sanitization and the temperature not result in modifications. Tzortzakis et al. (2007) described an absence of significant variance in vitamin C content in tomato after ozone treatment. Meanwhile, a decrease in the vitamin C content in guava, pineapple and banana after treatment with gaseous ozone has been observed (Allothman et al., 2010). In this study on 'Roxinho of Kenya', we expect that the treatment with ozonated water would induced significant alterations in the content of some antioxidants such as ascorbic acid. However, this result was not found in response to the ozone action, particularly in the fruit stored at room temperature, which showed lower durability.

The immersion of 'Roxinho of Kenya' passion fruit in ozonated water and stored at room temperature may have produced a detrimental effect on the antioxidant activity of the fruit at day 10 of storage (Table 5). In the refrigerated

Table 4. Ascorbic acid content (mg 100 g⁻¹) in 'Roxinho of Kenya' passion fruit ozonated and stored in two temperatures.

Storage (days)	Ascorbic acid (mg 100 g ⁻¹)					
	Room temperature			Cold chamber		
	Control	O ₃ -5min	O ₃ -10min	Control	O ₃ -5min	O ₃ -10min
0	49,02 ± 0,00 ^{Bc*}	57,60 ± 2,45 ^{Ab}	51,47 ± 2,83 ^{Ba}	49,02 ± 0,00 ^{Bc}	62,50 ± 2,45 ^{Ab}	51,47 ± 2,83 ^{Bb}
5	56,37 ± 2,83 ^{Aa}	50,25 ± 2,45 ^{Bc}	52,70 ± 2,45 ^{Ba}	51,47 ± 2,83 ^{Ac}	51,47 ± 2,83 ^{Ac}	52,70 ± 2,45 ^{Ab}
10	56,37 ± 2,83 ^{Ba}	61,27 ± 2,83 ^{Aa}	46,57 ± 2,83 ^{Cb}	55,15 ± 2,45 ^{Bb}	61,27 ± 2,83 ^{Ab}	52,70 ± 2,45 ^{Bb}
15	57,50 ± 2,89 ^{Aa}	50,00 ± 0,00 ^{Cc}	53,75 ± 2,50 ^{Ba}	58,75 ± 2,50 ^{Ba}	67,50 ± 2,89 ^{Aa}	58,75 ± 2,50 ^{Ba}
20	47,50 ± 2,89 ^{Ac}	48,75 ± 2,50 ^{Ac}	-	53,75 ± 2,50 ^{Bb}	58,75 ± 2,50 ^{Ab}	57,50 ± 2,89 ^{Aa}
25	52,50 ± 2,89 ^b	-	-	48,75 ± 2,50 ^{Ac}	47,50 ± 2,89 ^{Ad}	47,50 ± 2,89 ^{Ac}
30	-	-	-	57,50 ± 2,89 ^{Ba}	67,50 ± 2,89 ^{Aa}	48,75 ± 2,50 ^{Cc}

* Means followed by the same capital letter in the line and lowercase in the column do not differ between themselves by the Scott-Knott test at 5% of significance. O₃, ozone sanitization.

Table 5. Antioxidant activity of 'Roxinho of Kenya' passion fruit (%), stored in room temperature and cold chamber.

Storage (days)	Antioxidant activity					
	Room temperature			Cold chamber		
	Control	O ₃ -5min	O ₃ -10min	Control	O ₃ -5min	O ₃ -10min
0	77.52 ± 0.77 ^{Ae*}	71.91 ± 0.31 ^{Bc}	66.18 ± 0.83 ^{Cd}	76.37 ± 0.66 ^{Ac}	72.10 ± 0.88 ^{Bc}	64.50 ± 0.68 ^{Ce}
5	80.19 ± 0.76 ^{Ac}	69.81 ± 0.39 ^{Bd}	70.85 ± 0.61 ^{Bc}	82.47 ± 0.62 ^{Cb}	84.95 ± 0.19 ^{Ba}	88.57 ± 0.79 ^{Aa}
10	82.58 ± 1.02 ^{Cb}	88.19 ± 1.33 ^{Aa}	86.15 ± 1.06 ^{Ba}	70.88 ± 0.70 ^{Ae}	48.23 ± 0.87 ^{Cf}	57.48 ± 0.79 ^{Bg}
15	74.43 ± 0.45 ^{Bf}	71.04 ± 1.39 ^{Cc}	81.99 ± 0.98 ^{Ab}	77.30 ± 0.78 ^{Bc}	82.08 ± 0.56 ^{Ab}	71.25 ± 0.96 ^{Cc}
20	87.27 ± 0.75 ^{Aa}	82.10 ± 0.60 ^{Bb}	-	90.00 ± 1.02 ^{Aa}	49.60 ± 0.63 ^{Ce}	62.20 ± 0.84 ^{Bf}
25	79.01 ± 0.82 ^d	-	-	74.59 ± 0.51 ^{Ad}	68.89 ± 0.91 ^{Bd}	68.47 ± 0.85 ^{Bd}
30	-	-	-	76.58 ± 0.27 ^{Cc}	81.76 ± 1.11 ^{Ab}	78.78 ± 0.54 ^{Bb}

* Means followed by the same capital letter in the line and lowercase in the column do not differ between themselves by the Scott-Knott test at 5% of significance. O₃, ozone sanitization.

environment, the fruit showed higher antioxidant activity at the fifth day of storage, although presenting variations of the compounds on the other evaluation days.

The storage conditions to which the fruits and vegetables are subjected to is one of the determining factors for the post-harvest life. The temperature has a significant influence in the respiratory metabolism and in the microbial activity of these vegetables, directly determining its useful life. Thus, the use of refrigerated environments is one of the means of controlling the fruit respiration and the transpiration during the storage, reducing the respiratory rates, and slowing retarding fruit ripening (Rotili et al., 2013). In this study, the temperature was effective in maintaining the post-harvest life, even though the use of ozonated water did not cause an increase in antioxidants.

Conclusion

The use of ozonated water as a sanitizer is viable for 'Roxinho of Kenya' passion fruit. Immersion treatments for five minutes does not promote damages and maintains fruit quality. When considering fruit storage, a cold chamber must be used a cold chamber, in order to extend the post-harvest life and maintain the levels of antioxidant compounds.

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