



## Área: Ciência de Alimentos

# CAROTENOIDS AND PHYSICOCHEMICAL PROPERTIES OF 'DEKOPON' (*Citrus reticulata* Blanco Shiranuhi) JUICE

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**RESUMO** – ‘Dekopon’ presents physicochemical and phytochemical characteristics of great interest and, when processed, are consumed mainly in juice form. In this context, the aim of this study was investigate carotenoids and physicochemical properties of pasteurized and fresh ‘Dekopon’ juice. The results demonstrated that cold storage preserved more intensely the pasteurized juice characteristics than fresh juice. Thus, the pasteurized juice of ‘Dekopon’ demonstrates great potential to be used in the food industry.

**Palavras-chave:** Dekopon, juice, pasteurization, bioactive compounds.

## 1 INTRODUCTION

‘Dekopon’ tangerine (*Citrus reticulata* Blanco Shiranuhi) results of a crossing between ‘Kiyomi’ tangor (*Citrus unshiu* Marcov × *Citrus sinensis* Osbeck) and ‘Ponkan’ (*Citrus reticulata* Blanco) done in 1972 by Japanese Department of Agriculture. Therefore, ‘Dekopon’ tangerines belongs to genus *Citrus*, from family Rutaceae that includes about 17 species cultivated on tropical and temperate regions (KUMAR e BHASKAR, 2012). These tangerines show a parthenocarpic habit and produces almost seedless fruits, around 400 g of weight, very sweet and juicy. This variety is very well adapted to the climate in the highlands of São Paulo, where is also named as ‘Kinsei’ (LIM, 2012; MATSUMOTO, 2001).

‘Dekopon’ tangerine presents bioactive compounds such as carotenoids and flavonoids, which play different activities, including antioxidant, anti-inflammatory, antimicrobial and prevention of colon cancer (LIN et al., 2003; SUZUKI et al., 2004; YANG et al., 2009; YOSHIKAWA et al., 2006). Thus, ‘Dekopon’ can be considered a good source of health beneficial compounds which can be found in its flesh and peel.

The content of vitamin C, another important bioactive compound, is one of the reasons of high consuming of citrus fruit. In addition to its natural form, they are widely consumed as juice, which can be fresh or pasteurized, since customers are demanding by healthy and practice food with longer shelf life, sensory and nutritional characteristics preserved (DUTRA e LEMOS, 2012).

Although Brazil is the largest producer of citrus fruit, ‘Dekopon’ tangerine is still not widely known in the country, as well as its features. Thus, this study aimed to evaluate the physicochemical properties and bioactive compounds concentrations in ‘Dekopon’ juices (fresh and pasteurized) during cold storage.



## 2 MATERIAL AND METHODS

### *Sampling*

Fruits of 'Dekopon' tangerine (*Citrus reticulata* Blanco Shiranuhi) were acquired at São Paulo Supply Center (CEAGESP). Fruits were grown in Turvolândia (21° 52' 33" S, 45° 47' 13" W; MG/Brazil) at 840 m height, with humid subtropical climate. The fruits ( $\approx$  3 kg) were frozen and transported to Bioactive Compounds Laboratory of Food Science and Technology Institute at Federal University of Rio Grande do Sul (UFRGS, Porto Alegre/RS/Brazil).

The juice production was performed according to pre-established method, with small modifications (JACHNA et al., 2015). In summary, a portion of juice produced by a centrifuge (Walita® Juicer) was pasteurized (15 min at 85 °C) and the other portion remained fresh. Four samples of fresh juice and six samples of pasteurized juice were stored in amber glass flasks at  $7 \pm 2$  °C for 3 and 15 days, respectively. This difference in storage time was due to juices shelf life (fresh and pasteurized).

### *Juice physicochemical properties*

Fresh juice was evaluated immediately after preparation (day zero) and on days 1 and 3 after storage. Pasteurized juice was evaluated immediately after pasteurization (day zero) and after 1, 3, 7, 11 and 15 days of storage. The physicochemical parameters evaluated for both juices were reducing sugars, total soluble solids (TSS), total titratable acidity (TTA), acidity, color and vitamin C. The methodologies were carried out in triplicate according to Association of Analytical Communities methods (AOAC, 2005).

Reducing sugars: The values of reducing sugars, expressed in % of glucose, were determined by Fehling method.

Total soluble solids (TSS): Content of TSS was determined with a refractometer (Atago Co., Tokyo, Japan) and expressed in °Brix.

Total titratable acidity (TTA): Titration was utilized to quantify TTA and values were expressed in g of citric acid/100 mL.

Hydrogen potential (pH): pH was measured by pH-meter calibrated to pH 7 and pH 4 with standards solutions. Juices (5 g of each sample) were diluted in distilled water (80 mL) to obtain the measurement.

Color: Fresh and pasteurized juices were submitted to colorimetric evaluation (Konica Minolta Co., Osaka, Japan) to obtain the CIE LAB parameters, where *L* represents the brightness, *a\** colors varying from green to red and *b\** colors from blue to yellow.

Vitamin C: It was determined the proportion of L-ascorbic acid mixing 5 g of each sample juice, 3 mg of butylated hydroxytoluene (BHT) and 20 ml of 0.05 M sulphuric acid supra pure using an Ultra-Turrax for 1 min, followed by centrifugation at 4 °C at 25,400 x g for 15 min. Supernatant was then filtered in hydrophilic filter of Teflon and submitted to HPLC analysis using a C18 polymer column (250 mm x 4.6 mm ID, 5  $\mu$ m) (Vydac, Southborough, Massachusetts, USA), with 0.05 M sulphuric acid supra pure as mobile phase at 1.0 mL.min<sup>-1</sup> flow rate, injection volume of 10  $\mu$ L, and UV-visible detector set at 254 nm. Vitamin C quantification was determined comparing the value of sample peak with the peak obtained for a solution 1 g.L<sup>-1</sup> of L-ascorbic acid (ROSA et al., 2007).

### *Juice Carotenoids*

Carotenoids: The carotenoid content was evaluated on days zero, 1 and 3 for 'Dekopon' fresh juice and on days zero, 1, 3, 7, 11 and 15 for pasteurized juice. Extracts were prepared diluting each juice sample (3 g) in cold acetone, followed by successive washes with distilled water, petroleum ether and alcohol ether. Then, it was submitted to the saponification process adding 10% KOH in methanol during overnight (dark at room temperature). After that, it was washed and the extracts were concentrated in a rotary evaporator (Fisatom Quimis 0214 M2) (T < 35 °C) (MERCADANTE e RODRIGUEZ-AMAYA, 1991). Methyl tert-butyl ether (MTBE) was utilized to recover the samples and apply them to high performance liquid chromatography (HPLC) for quantitation. The analysis were carried out in triplicates and carotenoids were identified using a C30 reversed phase polymeric column (250 mm x 4.6 mm ID, 3  $\mu$ m) (YMC, Japan). The wavelength was adjusted to 450 nm. The elution gradient of mobile phase (consisting of MTBE/water/methanol) was set as follow: 5:90:5 to start, then 0:95:5 reached after 10 minutes, 0:89:11 at 20 minutes, 0:75:25 at 30 minutes and 0:50:50 at 40 minutes. The mobile phase flow rate was 1 mL min<sup>-1</sup>, injection volume was 5  $\mu$ L and the injector temperature was 33 °C (ZANATTA e MERCADANTE, 2007). Carotenoid quantitation was based on standard curves constructed with patterns acquired from Sigma-Aldrich:  $\beta$ -carotene (5–50 mg.mL<sup>-1</sup>),  $\alpha$ -carotene (2–25 mg.mL<sup>-1</sup>), lutein (1–65 mg.mL<sup>-1</sup>),  $\beta$ -cryptoxanthin (4–100 mg.mL<sup>-1</sup>), and zeaxanthin (1–40 mg.mL<sup>-1</sup>). The limits of quantitation (LOQ) and detection (LOD) were, respectively, for  $\beta$ -carotene,  $10.89 \times 10^{-5}$  g.kg<sup>-1</sup> and  $6.53 \times 10^{-5}$  g.kg<sup>-1</sup>; for  $\alpha$ -carotene,  $3.28 \times 10^{-5}$  g.kg<sup>-1</sup> and  $1.97 \times 10^{-5}$  g.kg<sup>-1</sup>; for lutein,  $1.15 \times 10^{-5}$  g.kg<sup>-1</sup> and  $6.9 \times 10^{-6}$  g.kg<sup>-1</sup>; for  $\beta$ -cryptoxanthin,  $3.51 \times 10^{-5}$  g.kg<sup>-1</sup> and  $2.11 \times 10^{-5}$  g.kg<sup>-1</sup>; and for zeaxanthin,  $1.59 \times 10^{-5}$  g.kg<sup>-1</sup> and  $9.56 \times 10^{-5}$  g.kg<sup>-1</sup>. The results were expressed in  $\mu$ g of carotenoid per g of juice sample.



### Statistical analysis

Results were analysed by ANOVA with Tukey's comparison test at 5 % of significance, using the software 10.0 (STATSOFT Inc., São Paulo, Brazil).

## 3 RESULTS AND DISCUSSION

### Juice physicochemical properties

The data of reducing sugars, TSS, TTA, pH and vitamin C are shown in Table 1. Reducing sugars content in fresh juice decreased from first day to day 3 of storage. However, pasteurized juice presented the same level of reducing sugars during the storage period, and the pasteurization process did not interfere in this content ( $\approx 2\%$  of glucose). The fresh and pasteurized 'Dekopon' juices showed increase in TSS content from one day at cold storage, reaching  $11.13 \pm 0.15$  °Brix (fresh juice) and  $11.40 \pm 0.00$  °Brix (pasteurized juice), these values were preserved until the end of storage (3 days for fresh, and 15 days for pasteurized juice). 'Salustiana' orange juice presented similar values of TSS ( $11.19 - 11.37$  °Brix) (ROUSSOS, 2011). However, the values found in this study was lower than the results found for 'Dekopon' fruits cultivated in Korea ( $12.50 - 14.40$  °Brix) and Japan ( $12.00 - 16.00$  °Brix), where it is named 'Shiranuhi' (MATSUMOTO, 2001). Comparing with other citrus juices, 'Dekopon' juice also revealed lower values of TSS. For example, Valencia orange fresh juice presented  $12.30$  °Brix and it was preserved during storage (FARNWORTH et al., 2001). Juices of 'Shamouti' and 'Salustiana' orange presented  $12.25$  and  $12.80$  °Brix, these content was maintained during 15 days of cold storage (DEL CARO et al., 2004).

Total titratable acid (TTA) was approximately  $0.65$  g citric acid/100 mL for fresh and pasteurized juices, and did not suffer variations by pasteurization process. During cold storage, the TTA value decreased to  $0.57\%$  g citric acid/100 mL after three days in fresh juice, and increased to  $0.71$  g citric acid/100 mL after 11 days in pasteurized juice. These data are very similar to those ( $1 - 1.2\%$  of citric acid) found by a research group that investigated the characteristics of 'Shiranuhi' from Japan (Matsumoto, 2001). It was reported for citrus juices of different fruits from Saudi Arabia TTA values of  $5.73$  (lemon juice),  $1.37$  (mandarin juice) and  $1.69\%$  (orange juice) of citric acid (AL-JUHAIMI e GHAFOR, 2013). Another research analysing juices from different varieties of lemon, mandarin and orange demonstrated results close to the current study,  $1.89 - 4.24$ ,  $0.72 - 1.30$ , and  $0.83 - 1.87\%$  of citric acid, respectively (ROUSSOS et al., 2013).

The juices pH was practically stable during storage with values from  $3.37 \pm 0.03$  to  $3.89 \pm 0.02$ . The minimum value was acquired for pasteurized juice immediately after pasteurization, and the maximum pH was assessed in fresh juice immediately after preparation. The pH of fresh and pasteurized juice showed not significantly differences from day 1 to the last day of storage. These pH values are similar to the work conducted with fruits grown in Turkey, the pH of tangerine (different varieties of *C. reticulata*) juice varied from  $3.17$  to  $3.69$  and of sweet orange (different varieties of *C. sinensis*) varied from  $3.22$  to  $3.48$  (Karadeniz, 2004). In other research about juices from different cultivars of oranges and mandarins was reported similar pH values ( $3.48$  to  $3.86$ ) (ROUSSOS et al., 2013).

Citrus juices attract the interest of consumers because of healthy benefits of its bioactive compounds, including carotenoids and vitamin C. In the present study, 'Dekopon' fresh juice presented  $393.91 \pm 14.60$  mg of L-acid ascorbic.kg<sup>-1</sup> of juice. This result was similar to the content demonstrated for orange fresh juices ( $361.50$  to  $408.50$  mg.L<sup>-1</sup> of juice) (Klimczak et al, 2007), but was higher than 'Eureka' lemon fresh juice ( $312.40$  mg.L<sup>-1</sup> of juice) (AL-JUHAIMI, 2014). However, 'Salustiana' orange fresh juices showed higher contents of vitamin C, varying from  $649.70$  to  $725.10$  mg.L<sup>-1</sup> of juice (ROUSSOS, 2011). It was reported that oranges present higher ascorbic acid content than tangerines (COUTO e AL-JUHAIMI, 2010), what can explain values found to 'Dekopon' fresh juice.

After the pasteurization process the vitamin C content was  $293.43$  mg of L-acid ascorbic.kg<sup>-1</sup> of juice, which is  $25.50\%$  less than the value found in fresh juice ( $393.91$  mg of L-acid ascorbic.kg<sup>-1</sup> of juice). However, from day 1 to day 3 of storage there was a decrease of  $21.69\%$  (fresh juice) and  $8.92\%$  (pasteurized juice) in the content of vitamin C. This demonstrates that pasteurization allows greater preservation of the vitamin C content of 'Dekopon' juice. Similar to fresh juice, the content of vitamin C in pasteurized juice decreased during the storage period, reaching  $183.62 \pm 13.67$  mg of L-acid ascorbic.kg<sup>-1</sup> after 15 days, which represents a decay of  $37.40\%$ . With or without pasteurization was observed vitamin C loss, and many factors can cause this loss of ascorbic acid, including time, temperature and environmental of storage (FARNWORTH et al., 2001; KLIMCZAK et al, 2007).

The vitamin C values found by this study are lower than those recorded for orange pasteurized juice ( $424.60$  to  $734.50$  mg.L<sup>-1</sup> of juice) (ESTEVE et al., 2005). The difference observed can be explained not only because of the species, but also due to differences in pasteurization process ( $77\text{ °C}$  for 20 seconds in the work developed by Esteve and collaborators /  $85\text{ °C}$  for 15 minutes in the present study).



**Table 1.** Values of pH, TTA, TSS, reducing sugars and vitamin C for fresh and pasteurized juices of 'Dekopon' tangerine stored for 0, 1, 3, 7, 11 and 15 days.

Days of storage	pH	
	Fresh Juice	Pasteurized Juice
0	3.89 ± 0.02 a A	3.37 ± 0.03 b B
1	3.64 ± 0.02 b A	3.61 ± 0.01 a A
3	3.67 ± 0.01 b A	3.61 ± 0.00 a A
7	-	3.56 ± 0.00 a
11	-	3.59 ± 0.05 a
15	-	3.53 ± 0.01 a
	TTA (g citric acid/100 mL)	
0	0.64 ± 0.00 a A	0.64 ± 0.01 b A
1	0.63 ± 0.01 a A	0.65 ± 0.01 b A
3	0.57 ± 0.01 b B	0.65 ± 0.01 b A
7	-	0.64 ± 0.00 b
11	-	0.71 ± 0.02 a
15	-	0.70 ± 0.01 a
	TSS (° Brix)	
0	10.87 ± 0.25 b A	10.55 ± 0.21 b B
1	11.13 ± 0.15 ab A	11.40 ± 0.00 a A
3	11.30 ± 0.10 a A	11.20 ± 0.14 a A
7	-	11.60 ± 0.00 a
11	-	11.35 ± 0.07 a
15	-	11.40 ± 0.00 a
	Reducing sugars (%)	
0	2.06 ± 0.03 a A	2.07 ± 0.06 a A
1	1.86 ± 0.03 b B	2.10 ± 0.05 a A
3	1.88 ± 0.02 b B	2.03 ± 0.07 a A
7	-	2.01 ± 0.03 a
11	-	2.04 ± 0.02 a
15	-	1.97 ± 0.03 a
	Vitamin C (mg of L-acid ascorbic.kg <sup>-1</sup> of juice)	
0	393.91 ± 14.60 a A	293.43 ± 21.15 a B
1	390.07 ± 2.64 a A	248.98 ± 20.54 ab B
3	308.46 ± 8.54 b A	267.27 ± 41.65 ab A
7	-	256.47 ± 23.63 ab
11	-	220.82 ± 0.01 ab
15	-	183.62 ± 13.67 b

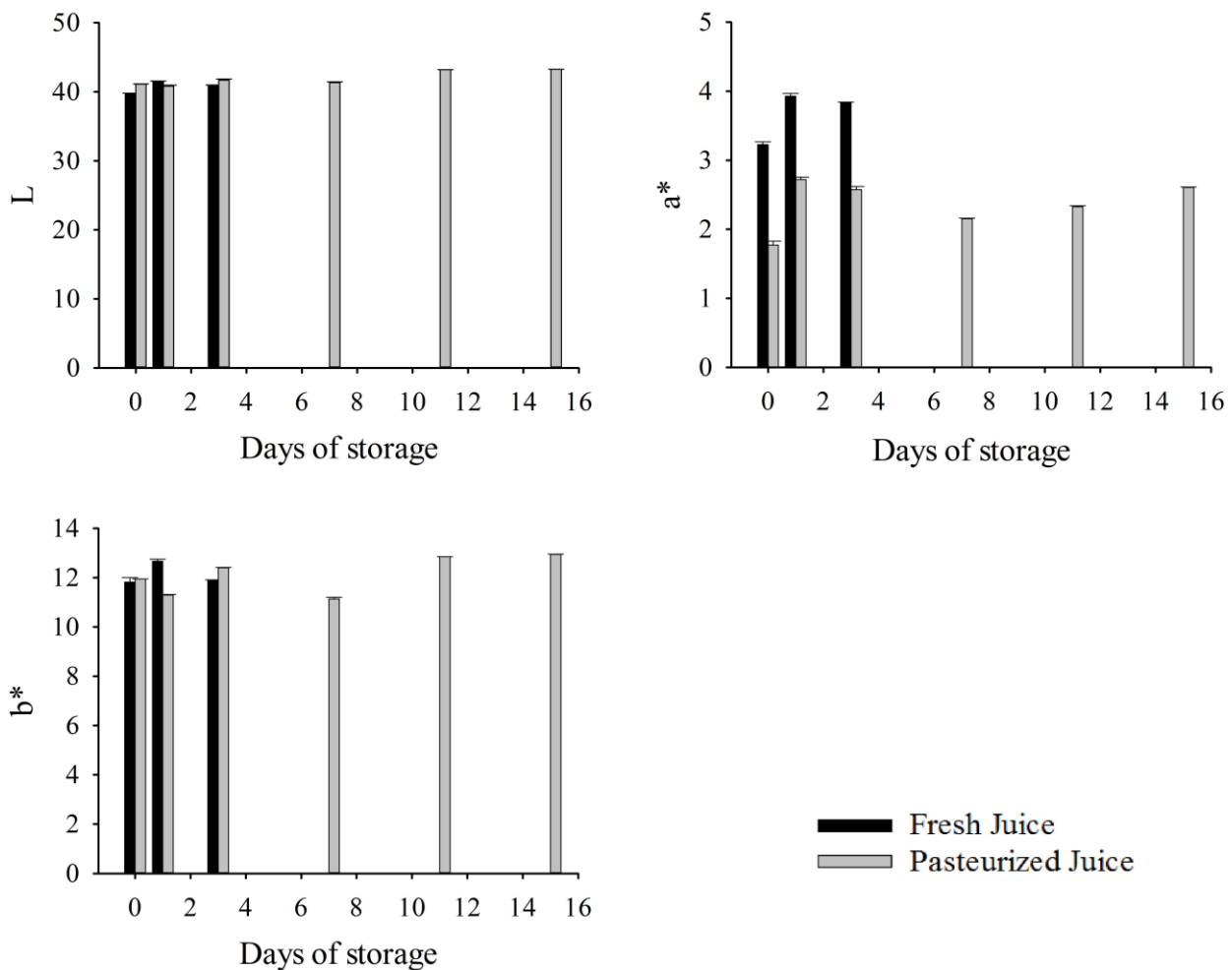
Mean ± standard deviation values followed by the same uppercase letter in line and by the same lowercase letter in column are not significantly different.

Color parameters  $L^*$ ,  $a^*$  and  $b^*$  (Figure 1) presented the same profile in fresh juice during cold storage, an increase in values from day 0 to day 1. In relation to L coordinate, pasteurized juices presented higher values than fresh juices during cold storage, due the oxidative prevention caused by the thermal process. After pasteurization the component  $a^*$  showed a considerable decrease when compared to fresh samples. It was observed the same behavior in orange juice treated by high intensity pulsed electric fields and pasteurization during refrigerated storage (CORTÉS et al., 2008). After 3 days of cold storage were observed higher values of  $b^*$  in pasteurized juice ( $12.40 \pm 0.02$ ) than in fresh juice ( $11.90 \pm 0.01$ ). With the pasteurization, it was also observed that 'Valencia' oranges showed changes in  $b^*$  values in a positive direction and changes in  $a^*$  values in a negative direction (LEE e COATES, 2003). Thus, a color change in positive directions ( $b^*$ ) and in negative directions ( $a^*$ ) indicates pasteurized citrus juice less reddish and more yellowish.





**Figure 1.** Color components (L, a\* and b\*) of fresh and pasteurized juices of 'Dekopon' tangerine stored for 0, 1, 3, 7, 11 and 15 days.



#### Juice Carotenoids

Carotenoids profile of 'Dekopon' juice demonstrated the presence of  $\beta$ -carotene (peak 4 of Figure 2), zeaxanthin (peak 2 of Figure 2), lutein (peak 1 of Figure 2) and  $\beta$ -cryptoxanthin (peak 3 of Figure 2), which was the major compound in both fresh and pasteurized juices. The profile obtained for pasteurized juice is shown in the chromatogram on Figure 2.

The individual and total carotenoids of fresh and pasteurized juice are shown in Figure 3. Total carotenoids in fresh juice decreased around 39.00 % in the first day and reached 51.20% after three days of cold storage. Similar decay was observed in the juice after the pasteurization process (51.70 %). However, after the pasteurization the content of carotenoids was preserved during all period of storage, providing an important feature to stability of pasteurized juice.

The individual carotenoids in 'Dekopon' juice followed the same decay profile of total carotenoids, and the higher lost was demonstrated by  $\beta$ -cryptoxanthin, the major compound, that lost more than 60.00 % after three days of storage, and 61.00 % after pasteurization.  $\beta$ -carotene showed the lowest decreasing, 20.70 % during fresh juice storage, and 22.20 % in the process of pasteurized juice. This carotenoid is well known because of its provitamin A activity (RODRIGUEZ-AMAYA, 2000).

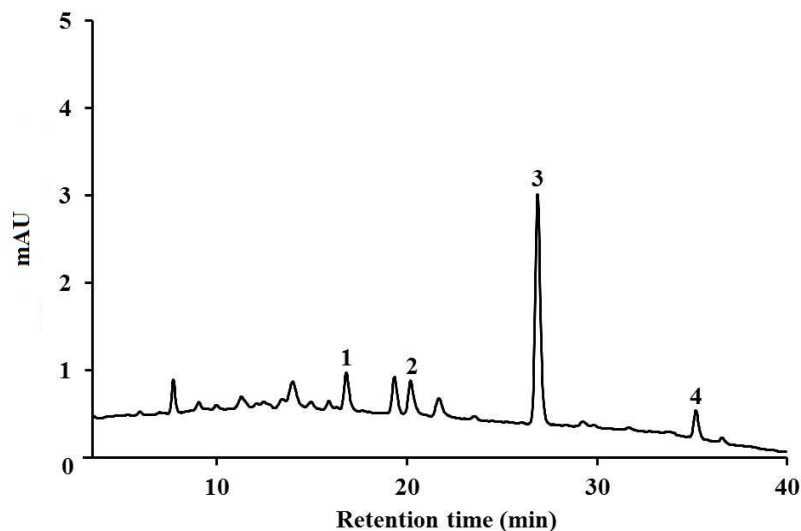
Lutein was the minor component and like  $\beta$ -carotene, it demonstrated small decay, 23.40 % in fresh juice during storage and 17.00 % after pasteurization. Lutein is an important compound for the eye healthy, due to its human health properties and the colorant feature, both lutein and  $\beta$ -carotene have been applied as food additive or supplement (AKHTAR e BRYAN, 2008).

The same compounds were found in 'Murcote' tangerine pasteurized juice (88 °C/30 seconds); however none of them presented a significant decreasing after the pasteurization process (DUTRA e LEMOS, 2012). Total carotenoids reduced only 11.80 %, reaching 14.2 mg kg<sup>-1</sup>, which value is almost 2-fold the content in 'Dekopon' juice. Observing



the compounds isolated,  $\beta$ -carotene was higher in 'Dekopon' ( $1.35$  and  $1.05 \text{ mg.kg}^{-1}$ ) than in 'Murcote' tangerine juice ( $0.96$  and  $0.93 \text{ mg.kg}^{-1}$ ) for both fresh and pasteurized juices.

**Figure 2.** Carotenoids profile of 'Dekopon' tangerine pasteurized juice immediately after preparation.

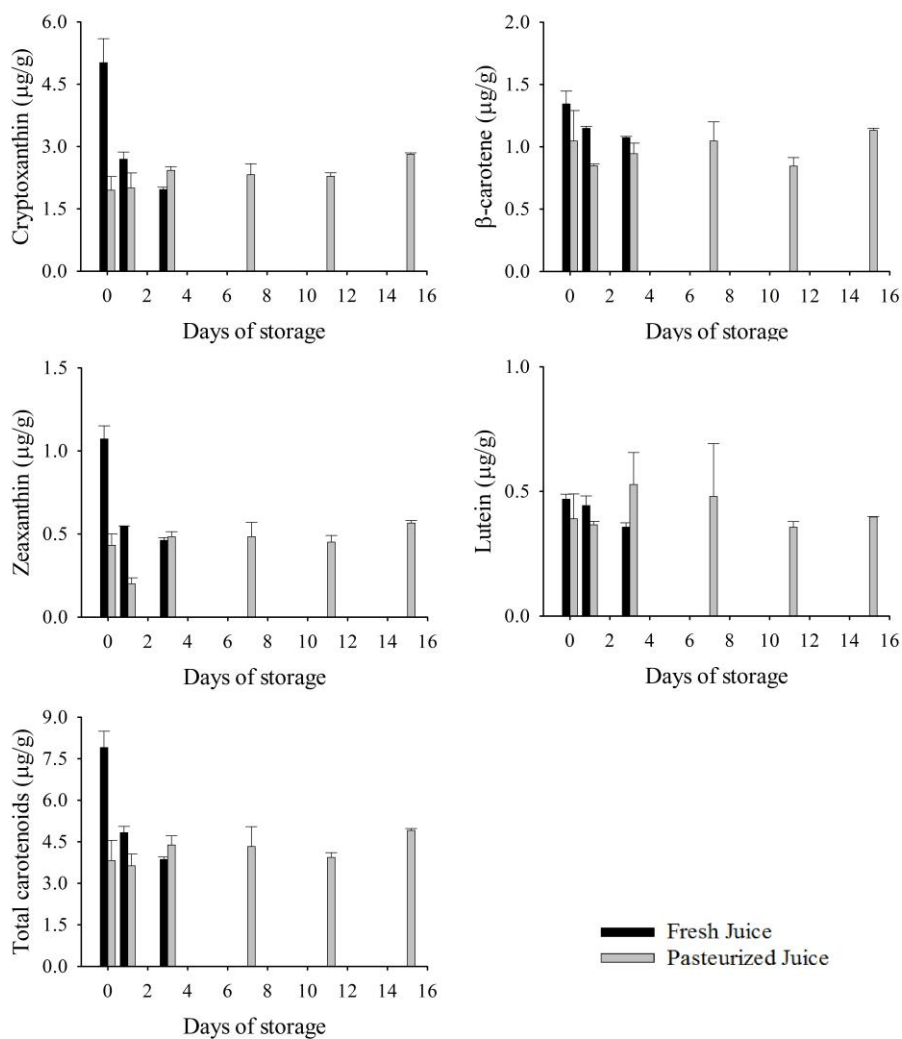


The temperature and time of pasteurization process influence the changes in the carotenoid content (MELÉNDEZ-MARTÍNEZ et al., 2007). In a study developed by Lee and Coates (2003) with 'Valencia' orange juice was observed that thermal treatments provide loss of carotenoids, especially on violaxanthin, antheraxanthin, and cisviolaxanthin. Juice of 'Brazilian Valencia' orange showed differences between the lutein content of fresh and pasteurized juice, a decrease of 20.86 % in lutein content of pasteurized juice (GAMA e SYLOS, 2007). In the current study, there was a decrease in lutein content after juice pasteurization (from  $0.47 \pm 0.02$  to  $0.39 \pm 0.10 \text{ mg kg}^{-1}$  juice), but without statistical difference. In the work of Gama and Sylos (2007), the zeaxanthin,  $\beta$ -cryptoxanthin and  $\beta$ -carotene contents did not change significantly after pasteurization. In contrast, in this experiment was noted significant change in the zeaxanthin and  $\beta$ -cryptoxanthin content immediately after thermal treatment.

It is important to highlight that carotenoids content, and consequently the fruit color, can presents variations even within the same citrus variety as a result of climatic characteristics from geographical localization of cultivar (MELÉNDEZ-MARTÍNEZ et al., 2007).



**Figure 3.** Content of individual and total carotenoids ( $\mu\text{g/g}$ ) in 'Dekopon' tangerine juices fresh and pasteurized stored for 0, 1, 3, 7, 11 and 15 days.



## 4 CONCLUSION

The characterization of 'Dekopon' tangerine juices showed that physicochemical properties has not undergone great changes after pasteurization process, and the cold storage preserved these features during storage. The fresh juice presented some decays in reducing sugars and TTA content. Concerning to the bioactive compounds, it was observed a decreasing in carotenoids content in fresh juice during the cold storage and after pasteurization, however, the pasteurized juice maintained the carotenoids content during the storage. Thus, the pasteurized juice of 'Dekopon' demonstrates great potential to be used in the food industry.



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