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Effect of chitosan coatings on the quality of persimmon under commercial storage conditions

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ABSTRACT

The chitosan coating effectiveness for enhancing the shelf life and retaining the carotenoids content of persimmons under commercial storage was investigated. Persimmons (*Diospyros kaki* L.) var. “Kyoto” were disinfected, immersed, drained, dried at room temperature, packaged and stored in a controlled temperature chamber. Physical and chemical properties were measured. The coating application reduced significantly the hue angle values compared with the control which suggests a slight ripening by the coating effect. Finally, the analysis of the carotenoid concentration for the five specific carotenoids determined that the β-cryptoxanthin was the most abundant pigment and it was not significantly differences during the storage and coating application. The storage time did not influence the carotenoid content for uncoated samples excluding the α-carotene analysis. The chitosan coating influenced on the lutein and zeaxanthin concentration during the last storage and on the α-carotene and β-carotene concentration for some cases.

KEYWORDS: HPLC, carotenoids, fruit, *Diospyros kaki* L.

INTRODUCTION

The persimmon is a climacteric fruit from Asia that is cultivated in some subtropical countries. In 2012, the main important producers were China (3.3 M tons), Republic of Korea (401,049 tons), Japan (253,800 tons) and Brazil (158,241 tons). The main countries of the Brazilian exportations are Netherlands, Canada, France and Portugal (FAO, 2012).

This fruit has an excellent sensorial acceptation, a high nutritional quality, a good taste and appearance. The principal damages during storage are caused by the excessive maturity, the firmness loss, the decaying and the peel browning. However, some techniques are developed to increase the storage life, prevent the losses and improve the marketing expansion (ROCHA; APARECIDA, 2006). The more important techniques are the storage under controlled conditions, the innovative packaging, the modified atmospheres, the edible films and coating, and others (SILVA *et al.*, 2011).

The chitosan (CH) (poly-(1,4)-2-amino-2-deoxy-P-D-glucose) has been used as a coating in fruits. It is obtained from the process of deacetylation of chitin (poly-(1,4)-2-acetamide-2- P-D- deoxy-glucose) by enzymatic or chemical methods. CH produces biodegradable, non-toxic and resistant coatings (THARANATHAN; KITTUR, 2003).

When chitosan coatings are applied on fruits, it has a good capacity to reduce the weight loss and the respiration rate (VALENCIA-CHAMORRO *et al.*, 2011). Additionally, it can maintain the firmness, register higher values of titratable acidity and lower values of pH as well as lower total soluble solid on coated fresh fruits compared to the control fruits (ADAY; CANER, 2010; DUAN *et al.*, 2011). The application of coatings on food prevents discoloration during storage, maintains the freshness and avoids the peel browning comparing with the control fruits (ANSORENA; MARCOVICH; ROURA, 2011; HOJO; DURIGAN; HOJO, 2011).

The persimmons are rich in polyphenols, vitamin C and carotenoid components such as β-cryptoxanthin, zeaxanthin, β,β-carotene and β,ε-carotene (ZHOU *et al.*, 2011) wich are considered as powerful antioxidants. The persimmons consumption can carry health benefits such as the reduction of degenerative human diseases, the protection against free radicals and the prevention of oxidative damage, risk of cardiovascular disease, diabetes and cancer (GIORDANI *et al.*, 2011). However, the antioxidant capacity and the carotenoid content of fruits are conditioned by the environmental factors and storage conditions which affect their nutritional value (BLESSINGTON *et al.,* 2010). Consequently, the importance of these studies is focused on the conservation of carotenoid on the food storage for promoting health and preventing human diseases (JAVANMARDI; KUBOTA, 2006).

The objective of this study was to investigate the effectiveness of chitosan coatings for enhancing the shelf life and retaining the carotenoids content of persimmons under commercial storage conditions.

MATERIALS AND METHODS

FRUITS

Persimmons *(Diospyros kaki* L.*)* var. "Kyoto" were harvested on mid-May, 2015, from Farroupilha, RS, Bazil located on 29°13'57"S 51°23'32"W. The fruits for the study were selected by size, absence of physical damage and at full orange color (grade 5 on the Japanese color chart) and transported to the Laboratório de Compostos Bioativos do Instituto de Ciência e Tecnologia de Alimentos da Universidade Federal do Rio Grande do Sul.

PREPARATION OF COATING SOLUTION

Chitosan with high degree of deacetylation (95%) and viscosity 74.03 cP was purchased from Polymar Indústria e Comércio LTDA, Fortaleza-CE, Brazil. The dissolution was prepared with CH in aqueous solutions (3%, w/v) of lactic acid to a final concentration of 1.0% v/v. It was mixed with glycerol (20% w/w) based in the chitosan weight using an Ultra-Turrax®.

COATING OF PERSIMMONS

Firstly the selected persimmons were sanitized with sodium hypochlorite solution (150 ppm) and following this, the fruits were divided into ten equal groups of three persimmons each one. Five groups were uncoated and were used as the control group and the remaining five groups were immersed completely in the edible coating solution of chitosan for 3 min. The excess coating solution on the fruits were drained and then dried at room temperature. The persimmons were placed into 20 x 30 cm polyethylene trays.

STORAGE AND PHYSICOCHEMICAL ANALYSIS CONDITIONS

They were packaged and were placed on the controlled storage conditions chamber at 5°C, 65% relative humidity (RH) during 14 days. From day 14th until day 18th all the groups of fruits were stored at 20°C with the objective of simulate the storage and marketing temperatures. The physicochemical analysis was done taking measurements straight away before storing, and on the days 7th, 14th, 16th and 18th after being stored. Each physicochemical analyses was taken for the coated and uncoated persimmons at the different evaluated time storage.

WEIGHT LOSS

Three persimmons were weighed during the study using a balance. The weight loss was calculated based on the reduction of the weight expressed as percentage loss of the initial weight.

TITRATABLE ACIDITY

Ten grams of the persimmons pulp and peel from three fruits were homogenized in 75 mL of distilled water using an Ultra-turrax®. Active coal was used to remove the color of samples. The samples were filtered and 50mL of this dissolution was titrated with 0.1 N sodium hydroxide. The results were expressed as a percentage of malic acid (PERSON, 1986).

SOLUBLE SOLID CONTENT (SSC)

Soluble solid content was measured from the dissolutions using a refractometer. The results were expressed in °Brix.

pH MEASUREMENTS

The pH of these dissolutions was determined for triplicate using a pH-meter (Q400MT, Quimis Aparelhos Científicos LTDA, São Paulo, Brazil).

FIRMNESS

Three persimmons were used for firmness analysis. Values were taken at five points on the circumference of each fruit. Firmness was measured with a Wheel Manual Test Stand (Model SLJ-B, CentMas Sdn Bhd, Kedah, Malaysia). The results were expressed in Newtons (N).

COLOR

Color was measured on the center of the flat surface and on the pulp of three fruits using a Minolta chroma meter (Model CR-400, Konica Minolta, Inc., Tokyo, Japan). The parameters L* (lightness), a* (greenness [−] to redness [+]) and b* (blueness [−] to yellowness [+]), hue angle (H) and chroma (C) values were recorded.

EXTRACTION AND ANALYSIS OF CAROTENOIDS AND VITAMIN A CONTENT

The carotenoid extract was prepared according to Mercadante, Rodriguez-Amaya (1998). This extract was transferred to petroleum ether, saponified with 10 % methanolic KOH, and storage at room temperature overnight. The alkali was removed with distilled water and the solvents were evaporated in a rotary evaporator. The extract was dried in a nitrogen flow and stored in the freezer until the quantification of carotenoids by high performance liquid chromatography (HPLC). Analyses were performed using a high performance liquid chromatography (HPLC) system equipped with a degasser, a quaternary pump solvent and detector UV / visible. The column used was a 250 mm x 4.6 mm i.d., 3 µm, C 30 reversed phase polymeric column (YMC, Japan). The mobile phase was water, 99.99% methanol (J.T.Baker, Mexico) and 99.96% tert-methyl butyl ether (MTBE) (J.T.Baker – Mallinckrodt, EUA) starting at 5:90:5 (v/v/v), reaching 0:95:5 (v/v/v) in 12 min, 0:89:11 (v/v/v) in 25 min, 0:75:25 (v/v/v) in 40 min and finally 0:50:50 (v/v/v) after a total of 60 min, with a flow rate of 1

mL/min at 33 °C (MERCADANTE; RODRIGUEZ-AMAYA, 1998). The carotenoids were quantified using the standard curves obtained for lutein (1–65 $mg.mL^{-1}$), zeaxanthin (1–40 $mg.mL^{-1}$), cryptoxanthin 4-100 $mg.mL^{-1}$ and β-carotene (5-50 $mg.mL^{-1}$). The results were expressed in micrograms per 100 g of sample. Vitamin A activity was calculated by the bioconversion factor following Guilland, Lequeu (1995). Yielding a value of 13 mg of b-carotene with 1 mg of retinol.

STATISTICAL ANALYSIS

Analysis of multiple-range tests were used to perform statistical analysis on all results, using Statgraphics® Centurion XV (StatPoint Technologies Inc, Warrenton, Virginia, USA). Differences between means were considered to be significant when $p \leq 0.05$.

RESULTADOS E DISCUSSÃO

WEIGHT LOSS

The loss weight during storage is produced due to transpiration and respiration processes that occur by the vapor pressure difference between fruit and air. This phenomenon causes significant economic losses during commercialization (VASILATOS; SAVVAIDIS, 2013).

The coatings used as a packaging can reduce the food weight loss (FALGUERA *et al.*, 2011). However, the results show that the chitosan coating did not reduce the weight loss. On the contrary the weight loss was higher for the coated persimmons on the 18th day (**Figure 1A**). The chitosan film is a semipermeable material to water vapor and this characteristic limits the food applications. Additionally, the acidity of the chitosan coating solution could produce damage on the fruit surface (ASSIS; ALVES, 2002, p. 5). Hojo, Durigan and Hojo (2011) and Santos et al. (2008) found that the increasing weight loss during storage of lychees and peaches was produced by the chemical reagents found in the commercial chitosan formulation as well as on the coating additives. However, most of researchers conclude that chitosan coating applications reduce the weight loss of food (JIANG; FENG; LI, 2012; PARK *et al.*, 2005).

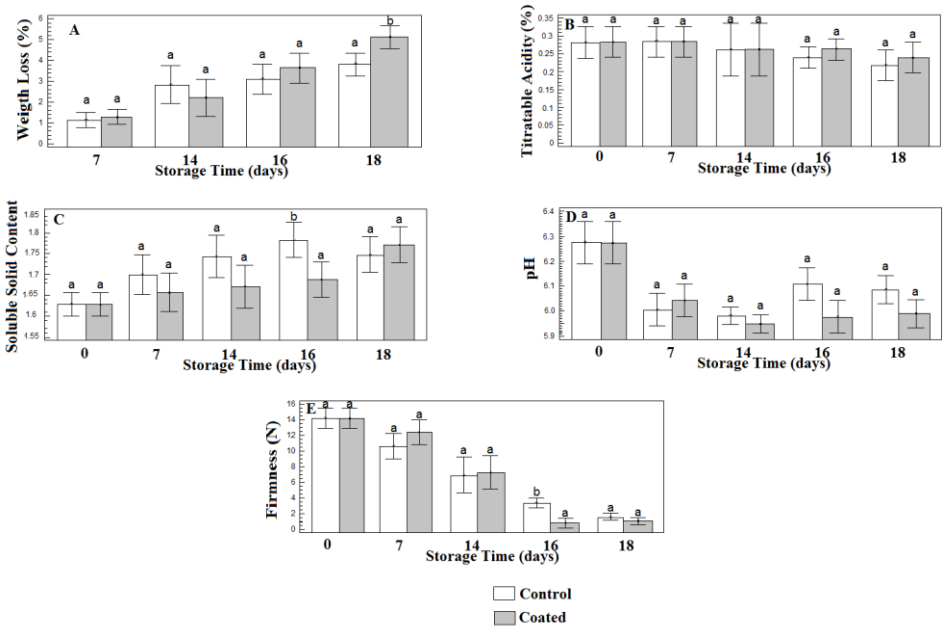

Figure 1. Evaluation of physicohemical properties of storage persimmons: A. Weight loss (%), B. Titratable acidity (% acid citric), C. Soluble solid content (°Brix), D. pH, E. Firmness (N). Lower-case letters highlight significance by Tukey's test ($p<0.05$).

TITRATABLE ACIDITY

The coating did not present a significantly influence on the total acidity (**Figure 1B**). However, others studies reported the total acidity reduction on the coated fruits during storage due to the fruit ripening what caused a organic acid consumption on the respiration (DANIELI *et al.*, 2002), for example using coatings on blueberries, strawberries, apples and lychees (DUAN *et al.*, 2011; HAN *et al.*, 2005; HOJO; DURIGAN; HOJO, 2011; SANTOS *et al.*, 2008).

SOLUBLE SOLID CONTENT (SSC)

The chitosan coating did not present a significant effect on SSC values on the majority of the cases (**Figure 1C**). However, significant differences were registered only on the 16th day, when the control persimmons recorded higher SSC values than the coated one which suggests a slight inhibition to ripening (ADAY; CANER, 2010). According to Murray, Valentini (1998), this property presents a high variability due to the sugar bioconversion, the soluble molecules formation on the cell wall, the balance of organic acid and the solubilization of salt. Similar results were found by others researches that used coatings to extend the shelf life of some fruits (ADAY; CANER, 2010; DUAN *et al.*, 2011; SILVA *et al.*, 2011).

pH MEASUREMENTS

This property is defined as the hydrogen activity in a logarithmic scale. The results did not show a significant effect on the coating pH values during the

storage (**Figure 1D**). However, a great hydrogen alteration has to occur to detect pH value differences (BLUM; AYUB; BARBOZA, 2009).

FIRMNESS

The firmness loss is produced by the degradation of insoluble pectins to more soluble pectins and to pectic acids. This phenomenon is caused by pectinesterase and polygalactronase activities that produce the shortening of chain length of the pectin.

The coating application delays the firmness loss rate due to a hydrostatic pressure increasing and the respiration rate decreasing (BLUM *et al.*, 2008; BLUM; AYUB; BARBOZA, 2009). However, there were no significant observed differences between coated and control persimmons in the majority of cases. On the contrary, the chitosan coating caused a significantly reduction of the persimmons firmness on the 14th day (**Figure 1E**). Similar results were obtained by other authors that did not observe significant differences on firmness of coated persimmons (CIA *et al.*, 2010; SILVA *et al.*, 2011).

COLOR

Color is one of the most important properties that consumer uses to judge the fruit maturity and quality. The control peel persimmons presented higher L* values than the coated persimmons only at the 0 day (**Table 1**). It could be produced by a non-homogeneous structure of the film on the fruit peel (FISK *et al.*, 2008). However, significant differences on the peel were not observed during storage. Similar results were reported by other authors, which suggest that L* of the peel was not affected significantly by the storage or coating on persimmons (CIA *et al*., 2010). However, the pulp lightness suffered a slightly reduction in some stages. The coated persimmons registered a lower lightness than the control group after the 14th day. It means that the coating application had a significant effect on the darkening of the pulp (SANTOS *et al.*, 2008).

Color changes are attributed to the ripening of fruits. It happens due to the chlorophyll reduction and the increasing of carotenes that produce higher values of a* and b*. Higher values of these properties on the peels and pulps were registered due to the coating application and the storage time (**Table 1**). It suggests a weak effect of the chitosan coating to retard the ripening process and thus it does not allow a significant extension of the persimmon shelf-life. However, other research found that the chitosan coating improves the fruit quality and appearance, avoids the darkening and retards the ripening for lychees, peaches and strawberries during storage (HAN *et al.*, 2005; HOJO; DURIGAN; HOJO, 2011; SANTOS *et al.*, 2008).

The chroma defines the color intensity or saturation represented in values near to zero for neutral colors as gray and near to 60 for intensive colors. It is represented as the distance of the hue angle from the center at the color tridimensional diagram. The chroma did not present significant differences for the persimmon peel and pulp during the storage time (CIA *et al.*, 2010). However, significant differences were observed on the peel at the 7 and 16 days (**Table 1**).

Table 1. Color of surface and pulp persimmons during storage. Means with different uppercase letters indicate a significant difference on the storage time effect (p≤0.05) and means with different lowercase letters indicate a significant difference on the coating effect (p≤0.05).

		Pulp		Surface	
	Days	Control	Coated	Control	Coated
L*	0	52.883^{a}_{B}	52.883^{a}_{C}	63.712^{b}_{A}	61.197^{a}_{A}
	7	47.112^{a}_{A}	49.254^{a}_{B}	64.287^{a}_{A}	63.079^{a}_{A}
	14	52.815^{a}_{B}	57.785^{b}_{D}	63.539^{a}_{A}	63.226^{a}_{A}
	16	48.51^{b}_{A}	42.889^{a}_{A}	63.296^{a}_{A}	61.577^{a}_{A}
	18	49.161^{b}_{AB}	41.24^{a}_{A}	63.236^{a}_{A}	61.505^{a}_{A}
a*	0	14.54^{a}_{B}	14.54^{a}_{A}	19.763^{b}_{AB}	16.447^{a}_{A}
	7	13.417^{a}_{B}	16.232^{b}_{A}	18.583^{a}_{A}	26.565^{b}_{A}
	14	11.715^{a}_{A}	15.704^{b}_{A}	21.045^{a}_{ABC}	26.808^{b}_{A}
	16	14.430^{a}_{B}	16.416^{a}_{A}	22.825^{a}_{BC}	27.776^{b}_{A}
	18	14.205^{a}_{B}	15.213^{a}_{A}	24.685^{a}_{C}	31.271^{b}_{B}
b*	0	36.729^{a}_{B}	36.729^{a}_{C}	65.127^{a}_{BC}	66.975^{a}_{B}
	7	32.841^{a}_{A}	33.279^{a}_{BC}	70.811^{a}_{D}	73.312^{a}_{C}
	14	30.999^{a}_{A}	30.437^{a}_{AB}	67.376^{a}_{C}	72.448^{b}_{C}
	16	31.145^{a}_{A}	30.144^{a}_{AB}	63.520^{b}_{AB}	58.591^{a}_{A}
	18	30.776^{a}_{A}	26.84^{a}_{A}	62.456^{b}_{A}	57.919^{a}_{A}
C*	0	39.576^{a}_{B}	39.576^{a}_{C}	68.124^{a}_{AB}	69.217^{a}_{B}
	7	35.520^{a}_{A}	37.054^{a}_{B}	73.321^{a}_{C}	78.097^{b}_{C}
	14	33.199^{a}_{A}	31.408^{a}_{A}	70.756^{a}_{BC}	77.476^{b}_{C}
	16	34.368^{a}_{A}	34.356^{a}_{A}	67.601^{a}_{A}	65.027^{a}_{A}
	18	33.963^{a}_{A}	30.917^{a}_{A}	67.361^{a}_{A}	66.221^{a}_{AB}
Hº	0	68.128^{a}_{BC}	68.128^{a}_{C}	73.176^{a}_{BC}	76.21^{a}_{C}
	7	67.785^{b}_{ABC}	63.986^{a}_{B}	75.419^{b}_{C}	70.154^{a}_{B}
	14	68.943^{a}_{C}	68.658^{a}_{C}	72.695^{a}_{BC}	69.72^{a}_{B}
	16	64.915^{b}_{ABC}	61.533^{a}_{AB}	70.284^{b}_{AB}	64.526^{a}_{A}
	18	65.084^{b}_{AB}	60.253^{a}_{A}	68.382^{b}_{A}	61.459^{a}_{A}

The hue angle of persimmons was located into the first quadrant. The color associated with more advanced ripening stage is the darker orange/red colors in contrast to yellow/oranges indicative of less ripe fruit (CIA *et al.*, 2010). The ripening produces the pigment synthesis like carotenoids which change the persimmons color (DONAZZOLO; BRACKMANN, 2002). A reduction of the hue angle values means an advanced ripening stage. The coating application reduced significantly the hue angle values compared with the control group during the days 7, 16 and 18. Additionally, the results showed a higher difference of hue angle values along the storage for the coated persimmons compared with the control group (**Table 1**). Different studies revealed opposite results which indicate the decrease of ripening by the coating effect on lychees, peaches and strawberries (HAN *et al.*, 2005; HOJO; DURIGAN; HOJO, 2011; SANTOS *et al.*, 2008). Although the chitosan coating did not delay the color changes, the darker orange color development on persimmons is associated with a ripe fruit which can improve the commercialization.

ANALYSIS OF CAROTENOIDS AND VITAMIN A

The most abundant carotenoid in the analyzed samples was the β-cryptoxanthin (ZHOU *et. al.*, 2011) (**Table 2**). However, others research showed that the β-carotene was the most abundant on the varieties Fuyu and Sheng (RODRIGUEZ-AMAYA, 1997, ZHOU *et al.*, 2011).

Table 2. Carotenoid content (mg/100 g dry weight) of persimmons *(Diospyros kaki* L.*)* var. "Kyoto" during storage to 18 days (two replicates).

Lutein		Zeaxanthin		β-cryptoxanthin		α-carotene	
Control	Coated	Control	Coated	Control	Coated	Control	Coated
99.44_{A}	99.44_{AB}	284.19_{A}	284.19_{AB}	682.72_{A}	682.72_{A}	32.57_{B}	32.57_{AB}
65.25^{a}_{A}	118.83^{a}_{B}	217.28^{a}_{A}	411.81^{a}_{B}	349.56^{a}_{A}	821.69^{a}_{A}	28.71^{a}_{B}	35.01^{b}_{B}
31.22^{a}_{A}	45.96^{a}_{A}	135.19^{a}_{A}	$182.1\,^{a}_{A}$	215.55^{a}_{A}	568.65^{a}_{A}	18.47^{a}_{A}	31.20^{b}_{AB}
61.30^{a}_{A}	53.46^{a}_{AB}	193.05^{a}_{A}	168.69^{a}_{A}	331.20^{a}_{A}	441.67^{a}_{A}	25.90^{a}_{AB}	32.0^{b}_{AB}
33.46^{a}_{A}	83.05^{b}_{AB}	147.08^{a}_{A}	305.51^{b}_{AB}	453.17^{a}_{A}	853.84^{a}_{A}	32.61^{a}_{B}	25.55^{a}_{A}

β-carotene		Total Carotenoid		Vitamin A	
Control	Coated	Control	Coated	Control	Coated
364.71_{A}	364.71_{AB}	1463.62_{A}	1463.62_{A}	$28{,}06_{A}$	$28{,}06_{A}$
306.96^{a}_{A}	481.18^{a}_{B}	967.76^{a}_{A}	1868.52^{a}_{A}	$23{,}61^{a}_{A}$	$37{,}01^{a}_{A}$
216.41^{a}_{A}	376.38^{b}_{AB}	616.83^{a}_{A}	1204.34^{a}_{A}	$16{,}65^{a}_{A}$	$28{,}95^{b}_{A}$
301.47^{a}_{A}	338.49^{a}_{A}	912.92^{a}_{A}	1034.37^{a}_{A}	$23{,}19^{a}_{A}$	$26{,}04^{a}_{A}$
314.24^{a}_{A}	405.59^{a}_{AB}	980.56^{a}_{A}	1673.53^{a}_{A}	$24{,}17^{a}_{A}$	$31{,}2^{a}_{A}$

with different uppercase letters indicate a significant difference on the storage time effect ($p \leq 0.05$) and with different lowercase letters indicate a significant difference on the coating effect ($p \leq 0.05$).

Additionally, the carotenoids content results were similar than in some many reports. In some cases, they were higher, for example the β-cryptoxanthin on the Fuyu and Sheng varieties (RODRIGUEZ-AMAYA, 1997). However, in some

exceptions, the reported values were higher in others research, for example the β-carotene on the Rama Forte variety (CARDOSO *et al.*, 2011), the zeaxanthin and the β-cryptoxanthin in others cultivars (ZHOU *et al.*, 2011), the α-carotene, the β-carotene and the cryptoxanthin on the Yotsumizo variety (RODRIGUEZ-AMAYA, 1997).

During ripening stages, the carotenoid content increase (RODRIGUEZ-AMAYA, 1997). However, the reduction in some stages could be caused by the low carotenogenesis rate on the advanced maturation stage or by herbicides that could have inhibited the carotenoids biosynthesis (DUTTA; CHAUDHURI; CHAKRABORTY, 2005). Simões et al. (2009) observed that carotenoids were slightly reduced during storage particularly in coated carrot sticks. Nevertheless, the results suggest that the chitosan coating contributes to maintenance of the carotenoid content. Additionally, in some cases the carotenoid content is higher for coating samples than the control. Consequently, the use of chitosan coating can significantly delay the oxidative stress in some cases for α-carotene, β-carotene and Vitamin A, and on the last storage stage for lutein and zeaxanthin.

CONCLUSION

Some physical and chemical properties are modified by the coating application during storage. Results suggest that the chitosan coating did not reduce significantly the persimmons weight loss neither increase significantly the firmness of the fruit during the storage By the other hand, titratable acidity and pH are not affected by coatings during storage. Additionally, the a* of the peel increases, the b* of the peel and pulp decrease and the L* of the peel is constant. Slightly higher carotenoids concentration is observed on the coated persimmons compared to the uncovered samples. Finally, the content of carotenoids has a different behavior during storage what suggests a low carotenogenesis rate and a significantly delay the oxidative stress in some specific cases.

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