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Additional Information

Effect of storage temperature on the crispness, colour and bioactive compounds of an orange snack obtained by freeze-drying

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Abstract

Purpose: A healthy and easy-to-use orange snack obtained from the freeze-dried orange pulp puree is proposed. Once the commercial packaging of the snack has been opened, the effect of conventional home storage temperature on its physicochemical properties and on the content of bioactive compounds has been studied. This research aims to recommend the consumer, and therefore the manufacturer, the best conditions for home storage of this product, keeping its nutritional quality and antioxidant capacity, as well as maintaining its color and crispness.

Design/methodology/approach: The water content, water activity, hygroscopicity, crispness, colour, vitamin C, β -carotene, total phenolic compounds, and antioxidant activity were characterised both when the orange snack was newly obtained and after one, two, and six months of storage inside zipper bags, at 4 and 20 °C.

Findings: The results indicated that, in these conditions, the orange snack increased its water content, causing a loss in both its porosity and its characteristic crispness. Nevertheless, the bioactive compounds remained stable throughout the storage period, with the exception of β -carotene, the content of which decreased markedly when the orange snack was stored at 20 °C.

Originality: Few studies have evaluated the stability of food products during home storage. The findings showed that the maximum storage time to ensure a proper texture of the orange snack studied is between 2 and 6 months, both at 4 and 20 °C. However, from the point of view of the conservation of both vitamin C and, especially, of β -carotene, it is recommended that this product be stored in refrigeration.

Keywords: crispness, colour, vitamin C, total phenolic compounds, β -carotene, zipper bag, orange puree, gum Arabic, bamboo fibre, antioxidant activity.

1. Introduction

Fruits have been reported to contribute positively to human health due to the antioxidant activity provided by different bioactive compounds, such as phytochemicals, some vitamins, and fibre (De Ancos et al., 2000). In particular, the orange and its derived products are a rich source of flavonoids (mainly hesperidin), carotenes, and vitamin C (Aschoff et al., 2015). Citrus fruit production is significant and is of great global economic importance, especially in the Mediterranean area. For this reason, fruit represents a good niche of opportunities for the development of new products and formats.

Orange is composed mainly of fibers, simple sugars and a wide variety of bioactive compounds that possess antioxidant properties and have a positive impact on health. Vitamin C, a characteristic compound of this fruit, is considered a powerful antioxidant that can inhibit the development of the main human oxidative reactions (Du et al., 2012; Zou et al. 2016). Phenolic compounds are also associated with a lower risk of suffering from different types of diseases. Most of these compounds, in citrus fruits, are flavonoids that include flavanones, flavones, and flavonols (Celano et al., 2019). Both hesperidin and narirutin are the predominant flavanones in orange (Hunlun et al., 2017) and have a wide spectrum of beneficial health effects involving the prevention of cancer and cardiovascular disease (Roohbakhsh et al., 2015) and the suppression of oxidative stress (Chanet et al., 2012). Nowak et al. (2018) showed that vitamin C and polyphenols act synergistically and define the antioxidant properties in both citrus and other fruits and vegetables. The carotenoids present in the orange, in addition to being responsible for the color and activity of provitamin A, probably have a relationship with the prevention and/or protection against cancer, heart disease, and macular degeneration, among other illnesses, which may be related in some way with their antioxidant properties (Meléndez-Martínez et al., 2007).

The snack market is one of the most rapidly evolving sectors. According to the Global Consumer Snacking Trends 'State of Snacking', 60% of adults prefer to eat small snacks during the day rather than larger meals, this number increasing to 70% for the 'millennials'. Also, 61% of the population can't imagine their life without daily snacking moments, which means that snacking is tightly integrated in the way we eat. In addition, snacking is considered a way of relating to each other, sharing identities and cultures (Mondelez International, 2019). Likewise, in recent years, consumers have become more and more aware of the importance of healthy food, increasing the demand for new healthy products. From this point of view, an orange snack obtained from the orange pulp puree by freeze-drying may be an attractive and ready-to-eat product for all ages. This orange snack may be consumed as such or may be crushed to obtain powdered fruit to be rehydrated and consumed as a juice or infusion, added to a dessert, dairy product, salad, ice cream, etc., and even for the purposes of enriching almost any food in bioactive compounds.

Freeze-drying is a dehydration technique known for providing final products of high nutritional and physical quality attributed to its less intense heating (An et al., 2016; Silva-Espinoza et al., 2020a). It has been reported that different biopolymers may be used as drying aids, in order to obtain more stable dried products, reducing the stickiness related to the low molecular weight sugar content in fruits (Telis & Martínez-Navarrete, 2012). In

this study, gum Arabic and bamboo fibre were used as carrier agents, since these biopolymers were observed to improve the final physical quality of the freeze-dried orange puree, reducing its hygroscopicity and increasing its glass transition temperature (Silva-Espinoza et al., 2020b). In addition, it has also been proven that they improve the in vitro bioaccessibility of the vitamin C and phenolic compounds present in the orange snack better than other biopolymers, such as maltodextrin, starch substituted with octenylsuccinic groups, native corn starch, or pea fibre (Silva-Espinoza et al., 2021).

However, it is important that the product maintain a good physicochemical quality as long as possible during storage. In addition, the stability of the different bioactive compounds throughout storage is a very important objective for obtaining a final orange snack with excellent nutritional and functional characteristics. Two types of storage may be considered: commercial storage and home storage. On the one hand, Silva-Espinoza et al. (2020b) suggested the maximum level of relative humidity (RH) in the environment which would preserve the crispy texture and the colour of the orange snack. The commercial packaging of this product may ensure that RH during marketing for long-time storage. However, it is also interesting to evaluate the stability of the orange snack during home storage, once the package is opened and considering the storage temperatures usually available at home: room or refrigeration temperature. The storage temperature has been observed to have an effect on the colour and functional compounds in dehydrated fruit and vegetables throughout storage (Cárcel et al., 2010; Miranda et al., 2014; Syamila et al., 2019).

The aim of this study was to evaluate the stability of the orange snack throughout 6 months of storage under simulated consumer home conditions inside zipper bags and at 4 and 20 °C. To this end, different physicochemical properties (water activity, water content, colour, texture, and hygroscopicity), bioactive compounds (vitamin C, total phenolic compounds, and β -carotene), and antioxidant activity were considered. With this research it is intended to recommend the consumer, and therefore the manufacturer, the best conditions for home storage of this product, keeping its quality as high as possible.

2. Material and Methods

2.1. Raw material

Oranges (*Citrus sinensis* cultivar 'Navelina') were purchased at a local supermarket in Valencia (Spain). They were selected based on a homogeneous size and colour, with no external physical damage.

As proposed by Agudelo et al. (2017) and Silva-Espinoza et al. (2020a), the biopolymers used as carrier agents were gum Arabic (GA, Scharlab, Sentmenat, Spain) and bamboo fibre (BF, VITACEL®, Rosenberg, Germany).

2.2. Sample preparation and storage

The orange pulp puree was triturated, mixed with (5 g GA + 1 g BF)/100 g orange puree, distributed in plates, 0.5 cm thick, and frozen at -45 °C (freezer chest Liebherr Mediline LGT 2325, Liebherr, Baden-Wurtemberg, Germany) for at least 24 hours. Then, the frozen samples were freeze-dried at 5 Pa, -45 °C in a condenser and at a shelf temperature of 50 °C for 5 h 50 min (Telstar Lyo Quest-55 freeze dryer laboratory

equipment, Telstar, Terrassa, Spain). Once the orange snacks were obtained, each of them was placed in a zipper bag (Albal®, Cofreso Ibérica S.A.U., Madrid, Spain) and all the air contained inside the bag was extracted manually. This procedure was selected for the storage study as it is a common way of storing this type of product in any home once its commercial packaging is opened. Nevertheless, for a better control, the zipper bags were distributed and placed into two hermetic canisters in which the relative humidity (RH) was 40% (TFA Dostmann GmbH & Co. KG, Wertheim, Germany), and were stored at 4 °C (Liebherr refrigerator GKv 6410 ProfiLine, Baden-Wurtemberg, Germany) and 20 °C (refrigerated cabinet Hotcold B, J.P. Selecta® S.A., Barcelona, Spain) for six months in darkness.

2.3. Analytical determinations

The orange snacks were analysed at different intervals: newly obtained after the freeze-drying (time 0 = t_0) and after one, two, and six months (t_1 , t_2 , and t_6), respectively.

2.3.1. Water content

The water content, (x_w g water/ 100 g orange snack), was determined using an automatic Karl Fisher titrator (Mettler Toledo, Compact Coulometric Titrator C10S, Worthington, OH, USA). Three replicates were taken per sample.

2.3.2. Water activity

The water activity (a_w) was measured with a dew point hygrometer (Aqualab 3TE, Meter Group, Munich, Germany). Two replicates were taken per sample.

2.3.3. Porosity

The porosity (ε , %), was calculated from the apparent density (ρ_a) and true density (ρ) according to Eq. (1). The ratio weight (g)/volume (cm^3) of five orange snack portions per sample was used to calculate the mean ρ_a (Eq. 2). These portions were obtained with a cylindrical punch and their height (h) and diameter (d) were measured with a Vernier calliper CM (0.02 mm; 1/1000"). ρ was calculated based on the sample composition (Eq. 3).

$$\varepsilon(\%) = 100 \frac{\rho - \rho_a}{\rho} \quad (1)$$

$$\rho_a = \frac{w}{\pi \left(\frac{d}{2}\right)^2 h} \quad (2)$$

where w , d , and h are the weight (g), height (cm), and diameter (cm) of each sample portion.

$$\rho = \frac{1}{\frac{x_w}{\rho_w} + \frac{x_{CH}}{\rho_{CH}}} \quad (3)$$

where x_w and x_{CH} are the mass fractions of the two main components of each sample (water and carbohydrates, respectively: x_w was determined as described in Section 2.3.1., and x_{CH} by difference); ρ_w and ρ_{CH} are their densities ($\rho_{CH} = 1.4246 \text{ g/cm}^3$, $\rho_w = 0.9976 \text{ g/cm}^3$ (Okos, 1986)).

2.3.4. Hygroscopicity

Hygroscopicity (H) was measured following the method of Goulas and Adamopoulos (2010), with minor modifications. Portions of each snack of the same size described in section 2.3.2. (~0.40 g) were placed at 25 °C in a hermetic plastic container filled with NaCl saturated solution (RH 75%). After 90 min, the portion was weighed, and H was expressed as g water gained / 100g orange snack solids. Three replicates were taken per sample.

2.3.5. Colour

The hue angle (h^* , Eq. 4) and chroma or saturation (C^* , Eq. 5) were calculated from the CIE L^* , a^* , and b^* colour coordinates measured on the surface of the orange snack (spectrophotometer Minolta, CM 3600D, Japan, reference system D 65, 10°). Five replicates were taken per sample. To calculate the global impact of each storage period and temperature on the colour of the orange snacks, Eq. (6) was used.

$$h^* = \arctan(b^*/a^*) \quad (4)$$

$$C^* = (a^{*2} + b^{*2})^{0.5} \quad (5)$$

$$\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2} \quad (6)$$

where ΔE^* is the total colour difference between the orange snack stored at each time and temperature, and that of the sample at t_0 .

2.3.6. Crispness

20 x 20 mm portions of the orange snack were penetrated with a cylindrical probe 10 mm in diameter, at 1 mms⁻¹ up to 80% strain, using a texture analyser (TA-XT2i, Stable Micro Systems, Godalming, UK). The force-distance curves were registered and the number of force peaks (force threshold 0.05 N) of each sample were obtained in order to evaluate the crispness of the orange snack. Four replicates were taken for each sample.

2.3.7. Vitamin C

The determination of the total vitamin C content (VC) was based on the reduction of the dehydroascorbic acid to ascorbic acid (AA) using high-performance liquid chromatography (HPLC) (Jasco, Italy). The reduction conditions were achieved out by using DL-dithiothreitol solution (Scharlab, Spain), taken as a reference to the procedure described by Silva-Espinoza et al. (2020a). The extraction was carried out according to Xu et al. (2008). The HPLC conditions were: Kromaphase100-C18, 5 mm (4.6 x 250 mm) column (Scharlau SL, Sentmenat, Spain); mobile phase 0.1% oxalic acid, volume injected 10 µL, flow rate 1 mL/min, detection at 243 nm (detector UV-visible MD-1510, Jasco, Cremella, Italy) at 25 °C. VC was identified by its retention time and quantified by the integration of the areas of the peaks obtained from the chromatograms using AA as standard. A standard solution of L (+) ascorbic acid (in the range of 5–200 ppm) was prepared (Scharlab SL, Sentmenat, Spain). The VC content was calculated as mg AA/100 g orange snack. This test was carried out in triplicate for each sample.

2.3.8. Total phenolic compounds

The method of Silva-Espinoza et al. (2020a) was followed for the methanolic extraction of total phenolic compounds (TP). The supernatant was collected and analysed as to TP using the Folin–Ciocalteu method, which was adapted from Singleton et al. (1999) with some modifications as described by Selvendran et al. (1990). The TP content was calculated as mg of gallic acid equivalents (GAE)/100 g orange snack. A standard curve in the range of 0–1000 ppm of gallic acid (Sigma-Aldrich, Saint Louis, MO, USA) was prepared. This test was carried out in triplicate for each sample.

2.3.9. Antioxidant activity

The methanolic extract obtained from the extraction of TP was used to evaluate the antioxidant activity (AOA) by means of FRAP and DPPH assays. The FRAP test was carried out by spectrophotometry according to Benzie and Strain (1999) and the absorbance was read at 593 nm. The DPPH test was also carried out by spectrophotometry, at 515 nm, following the method of Brand-Williams et al. (1995). The absorbance was measured at time 0 and after 15 min., when the reaction reached the steady state for the orange snack samples. The results were expressed in % DPPH following Eq. (7):

$$\%DPPH = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) * 100 \quad (7)$$

where A_{control} is the absorbance of the control (initial time) and A_{sample} the absorbance of the sample at the steady state.

The results for both methods were converted to mmol Trolox equivalents/100 g orange snack. Both tests were carried out in triplicate for each sample.

2.3.10. β -carotene

The β -carotene (BC) was extracted and quantified according to Igual et al. (2016), based on the use of a solution of hexane:acetone:ethanol (50:25:25, v/v/v) for extraction purposes and the spectrophotometric reference method of AOAC (2000) for the quantification. The absorbance was measured at 446 nm. The BC content was calculated as mg BC/100 g orange snack using a β -carotene (Dr. Ehrenstorfer, Augsburg, Germany) calibration curve in the range of 0.5–7 ppm. This test was carried out in triplicate for each sample.

2.3.11. Statistical analysis

Statgraphics Centurion XVII software was employed to perform a one-way analysis of variance (ANOVA) using Tukey's HSD test in order to establish the significant differences between samples with 95 % confidence interval ($p < 0.05$).

3. Results and Discussion

3.1. Physicochemical properties

The results obtained from the physicochemical evaluation of the orange snacks are shown in Table 1.

The water content of the orange snacks significantly ($p < 0.05$) and progressively increased from the first month of storage (Table 1). No significant effect of the storage temperature was observed at any time ($p > 0.05$) except at the end of the storage (t_6),

when the orange snack stored at 4 °C showed a lower water content ($p < 0.05$) than that stored at 20 °C. The increase in the water content may be a consequence of both the hygroscopicity of the orange snack and the permeability of the zipper bags. On the one hand, although the zipper bags are commercialised as impermeable storage food bags, they are usually made of low-density polyethylene, which may allow a slight permeability, so facilitating the interchange of gases and vapour. On the other hand, the H value of the newly-obtained orange snack was 6.5 ± 0.2 water gained / 100g orange snack solids. Similar values were obtained for tomato pulp and orange juice concentrate powders, which were evaluated using the same methodology as in this study and were qualified as evidently hygroscopic (Goulas & Adamopoulos 2008; Goulas & Adamopoulos 2010). Although the added biopolymers reduce hygroscopicity (Silva-Espinoza et al., 2020b), the low concentration of these in the formulation still makes the orange snack highly hygroscopic due to the hydrophilic groups present in the low molecular weight components and organic acids present in fruit. That increase in the water content caused, at the same time, the decrease in H, due to the fact that there was less availability for new bonds with water molecules from the environment, the difference being only significant at the end of storage at 20 °C, as compared with t_0 . (Table 1). On the other hand, the increase in the water content led to the progressive increase in the water activity ($p < 0.05$, Table 1) throughout storage. Water activity has been used as a common measure of stability of foods since it may be related to the relative rates of various deteriorative changes. Due to the composition of the orange snack, with the presence of polyphenols, ascorbic and citric acid, simple sugars, and free amino acids, and taking into account the low-intermediate a_w values shown by all the studied samples, especially mechanical changes and/or browning reactions may be expected to occur in this product. Consequently, the evolution of the texture and colour of the samples during storage was studied. The structure of the orange snacks was affected throughout storage. From the force-distance curves, the number of force peaks was obtained. They decreased as storage progressed, with no effect of the storage temperature. A much smaller number of force peaks were obtained from t_2 (Table 1, $p < 0.05$). This decrease may be related to the fact that the orange snacks lose part of their crispy behaviour (Alonzo-Macías et al., 2004). At the end of storage, no force peaks were observed at any storage temperature, which indicated the total loss of crispness at that moment. From this point of view, the decrease in crispness observed throughout storage time may be related to the evolution in the water content of the samples (Katz & Labuza, 1981). A previous study that relates the sorption isotherm of this freeze-dried orange snack with its mechanical properties indicates that storage at atmospheres with $RH \geq 32\%$, which supposes an $a_w \geq 0.32$ in the equilibrated sample, leads to the loss of its crispness (Silva-Espinoza et al., 2020b). In this sense, despite the small number of force peaks observed at t_2 , the crispness of these samples should still be considered acceptable. On the other hand, a decrease in the ε of the orange snacks was only observed at t_3 (Table 1, $p < 0.05$), although storage at 4 °C avoids a more pronounced loss of ε than at 20 °C ($p < 0.05$). A significant negative relationship was obtained between both the number of force peaks and ε with x_w ($r = -0.89$ and $r = -0.96$, respectively; $p < 0.001$), which indicates that the higher the water content, the lower the degree of crispness and porosity of the snack. Also, a significant positive correlation ($r = 0.67$, $p < 0.001$) was obtained between ε and the number of force peaks.

Table 1. Mean values (\pm standard deviation) of the physical chemical properties studied for the newly freeze-dried orange snack (t_0) and throughout storage (one month (t_1), two months (t_2), and six months (t_6) from the beginning of the storage), at 4 and 20 °C.

	t_0		t_1		t_2		t_6	
	-	4	20	4	20	4	20	
x_w	3.96 ± 0.18^d	4.10 ± 0.17^d	4.27 ± 0.06^d	5.85 ± 0.07^c	5.55 ± 0.07^c	8.4 ± 0.3^b	9.600 ± 0.001^a	
a_w	0.232 ± 0.002^f	0.246 ± 0.002^e	0.252 ± 0.001^d	0.301 ± 0.002^b	0.287 ± 0.002^c	0.415 ± 0.004^a	0.416 ± 0.002^a	
ε	85.7 ± 0.2^a	86.5 ± 0.2^a	85.3 ± 0.8^a	85.7 ± 0.6^a	85.0 ± 0.5^a	78.2 ± 1.9^b	74.5 ± 1.9^c	
H	6.5 ± 0.2^a	5.0 ± 0.3^a	4.2 ± 0.6^{ab}	4.4 ± 0.9^{ab}	4.5 ± 0.3^{ab}	3.7 ± 1.6^{ab}	1.5 ± 0.2^b	
L^*	84.9 ± 0.8^a	85.4 ± 0.9^a	84.2 ± 0.8^a	84.8 ± 0.9^a	84.1 ± 1.5^a	85.1 ± 0.5^a	81.7 ± 0.7^b	
C^*	38.8 ± 1.3^a	32.1 ± 1.6^c	37.6 ± 1.2^{ab}	38.4 ± 0.7^a	34.2 ± 1.3^c	34 ± 3^{bc}	40.4 ± 0.7^a	
h	86.7 ± 1.4^b	87.6 ± 0.3^a	86.7 ± 0.5^b	86.9 ± 0.2^{ab}	86.7 ± 0.6^b	86.9 ± 0.4^{ab}	84.4 ± 0.2^c	
ΔE	-	6.4	1.4	0.5	4.7	4.5	3.9	
Peaks	46 ± 4^a	47 ± 4^a	43 ± 8^a	9.8 ± 0.9^b	10 ± 6^b	0 ± 0^c	0 ± 0^c	

x_w : water content ($\text{g}_{\text{water}} / 100 \text{g}_{\text{orange snack}}$); a_w : water activity; ε : porosity (%); H: Hygroscopicity ($\text{g}_{\text{gained water}} / 100 \text{g}_{\text{orange snack solids}}$); L^* : luminosity; C^* : chroma; h: hue angle; ΔE : total colour difference as related to t_0 ; Peaks: number of force peaks

All these results suggest that orange snacks should be stored in $RH < 30\%$ to avoid the structural collapse reflected in a porosity decrease and a loss of crispness. In this sense, the proposed storage inside zipper bags allows the crispness of the snack to be ensured for a period of time of more than 2 but less than 6 months, at any of the studied temperatures.

The colour is another relevant physical property to be controlled since it is the first attribute that the consumers perceive. The values of L^* and h remained constant up to t_2 at both temperatures (Table 1). However, a small although significant decrease in L^* and h ($p < 0.05$) was observed in the sample at t_3 stored at $20\text{ }^\circ\text{C}$. Therefore, a slightly darker and less yellowish orange snack was shown by the sample after 6 months of storage at $20\text{ }^\circ\text{C}$, which may be a consequence of the gradual non-enzymatic browning which can begin to occur from a certain water content and water activity in the samples. It has been reported that the refrigerated storage of apple and citrus juices reduces product browning (Burdurlu & Karadeniz, 2003; Roig et al., 1999). Factors such as temperature, moisture, a_w , carbonyl compounds, organic acids, O_2 , and sugars are responsible for the non-enzymatic browning in stored foods (Muralikrishna et al., 1969). No clear trend in the evolution of C^* was observed as the storage progressed (Table 1). In any case, the global impact of each storage period and each temperature on the colour of the orange snacks may be more clearly observed through the total colour differences (Eq. 6), calculated with reference to the orange snack at t_0 . All the ΔE^* values obtained were lower than 6.4 (Table 1). It can be considered that ΔE^* values lower than about 6 units indicate small changes in colour (Mosquera et al., 2011; Telis & Martínez-Navarrete, 2009), which means that the length and temperature of storage had little impact on the colour.

3.2. Bioactive compounds and antioxidant activity

The impact of the storage period and temperature on the bioactive compound content and the antioxidant activity was evaluated. Fig. 1 shows the evolution of VC and TP. It can be observed that the VC content of the orange snack remained stable during storage at both temperatures, except after 6 months of storage at $20\text{ }^\circ\text{C}$, when a significant decrease was observed ($p < 0.05$). The decrease may be related to the higher a_w value of this sample, providing a greater availability of water to participate in degradative reactions. Moraga et al. (2012) also observed a significant decrease in the VC of grapefruit powder with a_w higher than 0.4 stored at $20\text{ }^\circ\text{C}$. Nevertheless, according to the results in this study, a lower storage temperature ($4\text{ }^\circ\text{C}$) seems to delay the degradation reactions, promoting the whole preservation of VC.

The TP content increased as the storage progressed (Fig. 1). Regardless of the storage temperature, higher values of TP content, were obtained, especially from t_2 ($p < 0.05$). The increase in TP content may be related to the synthesis of compounds with polyphenolic activity. It has been reported that the decrease in some organic acids during the storage period of citrus is due to their function as a substrate for the synthesis of phenolics, including anthocyanin and non-anthocyanin phenolics (Igual et al., 2010; Kalt et al., 1999).

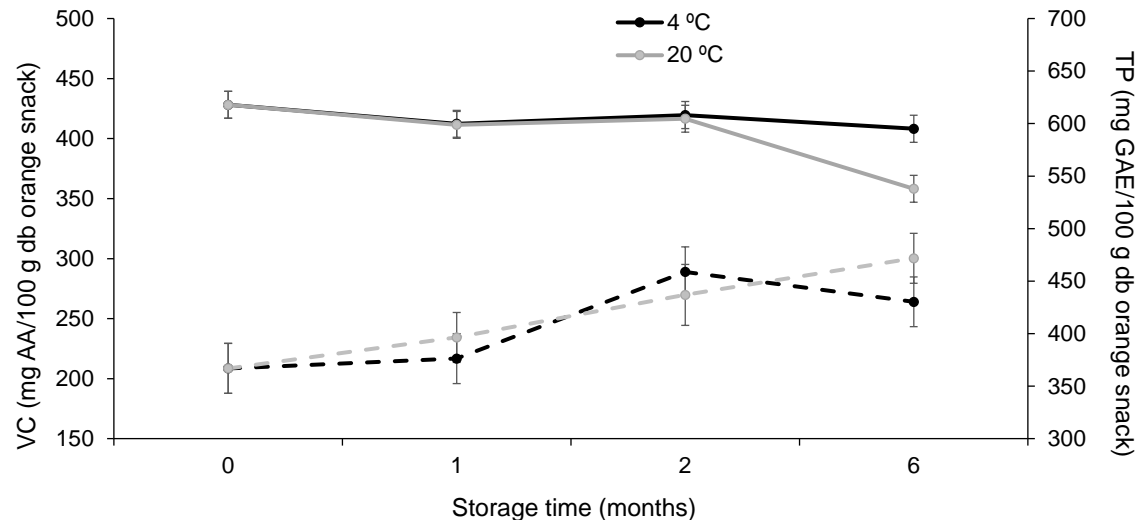


Figure 1. Evolution of vitamin C content (VC, left axis, solid lines) and total phenolic compounds (TP, right axis, dashed lines) throughout the storage period (storage time=0: newly-obtained orange snack) at 4 and 20 °C. Error bars represent Tukey's HSD statistical significance bars.

The BC content decreased significantly ($p < 0.05$) in the first month of storage, this being more pronounced when the orange snack was stored at 20 °C (Fig. 2). It indicated the fast degradation of this compound, despite the low a_w of the samples at that moment, due to the fact that it is extremely sensitive to oxygen and higher storage temperatures (Çinar, 2004; Leskova et al., 2006; Tang & Chen, 2000).

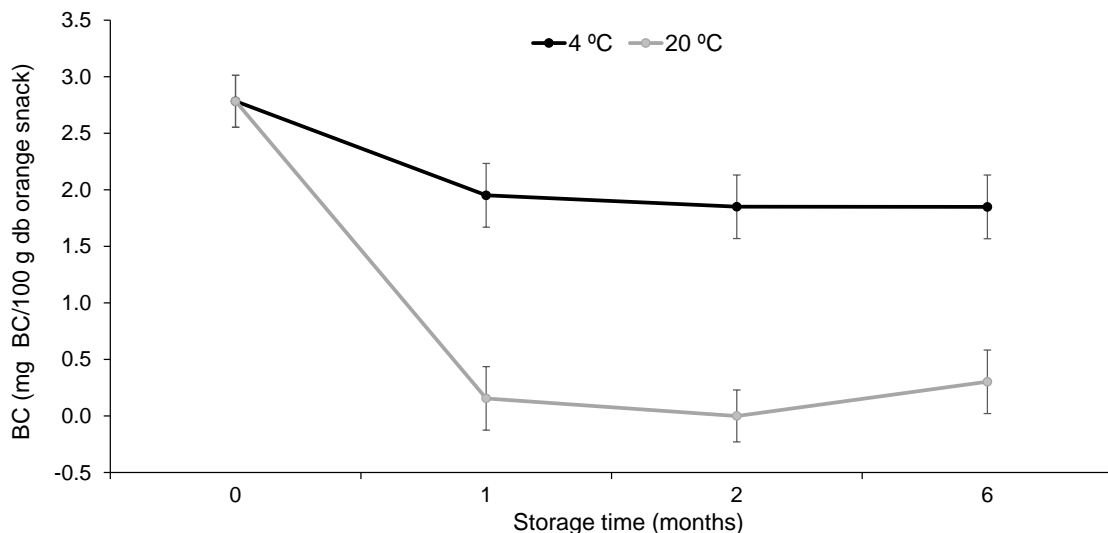


Figure 2. Evolution of β -carotene content (BC) throughout the storage period (storage time=0: newly-obtained orange snack) at 4 and 20 °C. Error bars represent Tukey's HSD statistical significance bars.

In general, the AOA remained stable during the whole storage period ($p>0.05$, Fig. 3), with values from $t_0=1.89$ to $t_6=1.84$ and 1.74 mmol Trolox equivalents/100 g orange snack at 4 and 20 °C, respectively, according to the DPPH assay. In the same way, the AOA obtained by the FRAP assay showed values from $t_0=2.0$ to $t_3=2.2$ and 2.1 mmol Trolox equivalents/100 g orange snack at 4 and 20 °C, respectively ($p>0.05$). The AOA is provided by different compounds, such as VC, carotenoids, and flavonoids, among others. Therefore, the high degree of stability of VC and TP obtained in this study may also have contributed to the markedly stable nature of AOA. Furthermore, according to the results of this study, the storage temperature did not seem to have a great impact ($p>0.05$) on the evolution of the AOA.

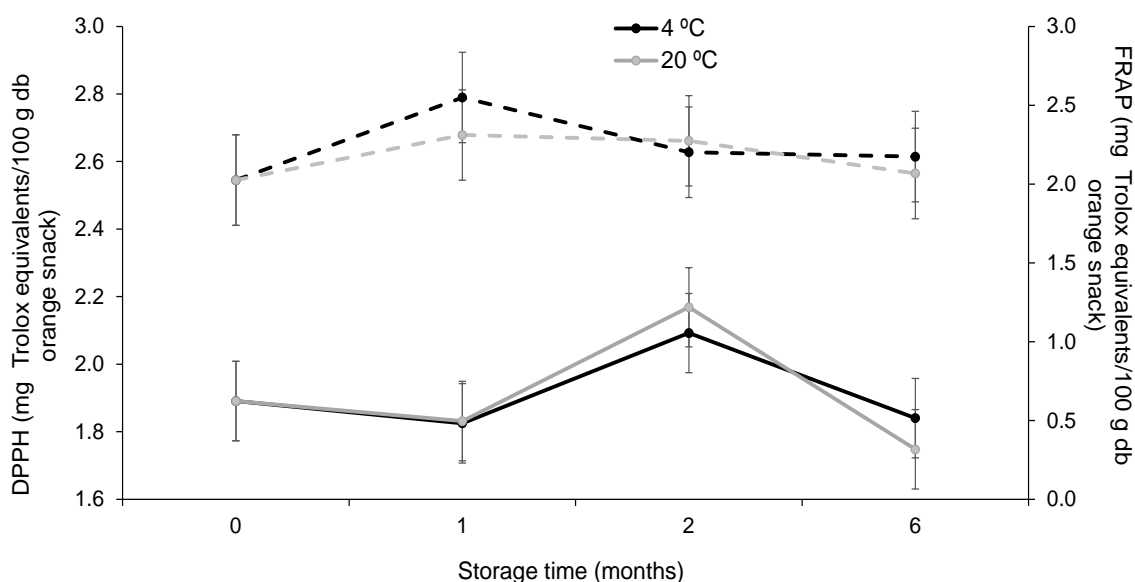


Figure 3. Evolution of the antioxidant activity measured by DPPH test (left axis, solid lines) and FRAP assay (right axis, dashed lines) throughout the storage period (storage time=0: newly-obtained orange snack) at 4 and 20 °C. Error bars represent Tukey's HSD statistical significance bars.

4. Conclusion

More than the colour change or the bioactive compound loss, the crispness is the critical property that defines the loss in quality of the orange snack during storage. Despite the use of zipper bags, the sample gained water as the storage time lengthened, with a consequent loss in porosity and crispness. In these conditions, a proper texture of the orange snack may be ensured for at least 2 months, both at 4 and 20 °C. However, from the point of view of the conservation of vitamin C and, especially, of β -carotene, the refrigerated storage of this product is recommended.

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Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

References

- Agudelo, C., Igual, M. M., Camacho, M. M., & Martínez-Navarrete, N. (2017). Effect of process technology on the nutritional, functional, and physical quality of grapefruit powder. *Food Science and Technology International*, 23(1), 61–74. <https://doi.org/10.1177/1082013216658368>
- Alonzo-Macías, M., Montejano-Gaitán, G., & Allaf, K. (2014). Impact of drying processes on strawberry (*Fragaria var. camarsosa*) texture: identification of crispy and crunchy features by instrumental measurement. *Journal of Texture Studies*, 45, 246–259. <https://doi.org/10.1111/jtxs.12070>
- An, K., Zhao, D., Wang, Z., Wu, J., Xu, Y., & Xiao, G. (2016). Comparison of different drying methods on Chinese ginger (*Zingiber officinale* Roscoe): Changes in volatiles, chemical profile, antioxidant properties, and microstructure. *Food Chemistry*, 197, 1292–1300. <https://doi.org/10.1016/j.foodchem.2015.11.033>
- AOAC (2000). *Official Methods of Analysis of AOAC International*, 17th ed. AOAC International.
- Aschoff, J. K., Kaufmann, S., Kalkan, O., Neidhart, S., Carle, R., & Schweiggert, R. M. (2015). In vitro bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices [*Citrus sinensis* (L.) osbeck]. *Journal of Agricultural and Food Chemistry*, 63(2), 578–587. <https://doi.org/10.1021/jf505297t>
- Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 299(1995), 15–27. [https://doi.org/10.1016/S0076-6879\(99\)99005-5](https://doi.org/10.1016/S0076-6879(99)99005-5)
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Burdurlu, H. S., & Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry*, 80(1), 91–97. [https://doi.org/10.1016/S0308-8146\(02\)00245-5](https://doi.org/10.1016/S0308-8146(02)00245-5)
- Cárcel, J. A., García-Pérez, J. V., Sanjuán, N., & Mulet, A. (2010). Influence of pre-treatment and storage temperature on the evolution of the colour of dried persimmon. *LWT - Food Science and Technology*, 43(8), 1191–1196. <https://doi.org/10.1016/j.lwt.2010.04.011>
- Celano, R., Campone, L., Pagano, I., Carabetta, S., Di Sanzo, R., Rastrelli, L., Piccinelli, A.L. & Russo, M. (2019). Characterisation of nutraceutical compounds from different parts of particular species of *Citrus sinensis* 'Ovale Calabrese' by UHPLC-UV-ESI-HRMS. *Natural Product Research*, 33(2), 244-251. <https://doi.org/10.1080/14786419.2018.1443102>
- Chanet, A., Milenkovic, D., Manach, C., Mazur, A. & Morand, C. (2012). Citrus flavanones: What is their role in cardiovascular protection?. *Journal of Agricultural and Food Chemistry*, 60(36), 8809–8822. <https://doi.org/10.1021/jf300669s>

- Çinar, I. (2004). Carotenoid pigment loss of freeze-dried plant samples under different storage conditions. *LWT - Food Science and Technology*, 37(3), 363–367. <https://doi.org/10.1016/j.lwt.2003.10.006>
- De Ancos, B., Gonzalez, E. M., & Cano, M. P. (2000). Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agricultural and Food Chemistry*, 48(10), 4565–4570. <https://doi.org/10.1021/jf0001684>
- Du, J., Cullen, J. J. & Buettner, G. R. (2012). Ascorbic acid: Chemistry, biology and the treatment of cancer. *Biochimica et Biophysica Acta - Reviews on Cancer*, 1826(2), 443–457. <https://doi.org/10.1016/j.bbcan.2012.06.003>
- Goula, A. M., & Adamopoulos, K. G. (2008). Effect of maltodextrin addition during spray drying of tomato pulp in dehumidified air: II. Powder properties. *Drying Technology*, 26, 726–737. <https://doi.org/10.1080/07373930802046377>
- Goula, A. M., & Adamopoulos, K. G. (2010). A new technique for spray drying orange juice concentrate. *Innovative Food Science and Emerging Technologies*, 11(2), 342–351. <https://doi.org/10.1016/j.ifset.2009.12.001>
- Hunlun, C., de Beer, D., Sigge, G. O. & Wyk, J. Van. (2017). Characterisation of the flavonoid composition and total antioxidant capacity of juice from different citrus varieties from the Western Cape region. *Journal of Food Composition and Analysis*, 62, 115–125. <https://doi.org/10.1016/j.jfca.2017.04.018>
- Igual, M., García-Martínez, E., Camacho, M. M., & Martínez-Navarrete, N. (2010). Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Food Chemistry*, 118(2), 291–299. <https://doi.org/10.1016/j.foodchem.2009.04.118>
- Igual, M., García-Martínez, E., Camacho, M. M., & Martínez-Navarrete, N. (2016). Stability of micronutrients and phytochemicals of grapefruit jam as affected by the obtention process. *Food Science and Technology International*, 22(3), 203–212. <https://doi.org/10.1177/1082013215585417>
- Kalt, W., Forney, C.F., Martin, A., & Prior, R. L. (1999). Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of Agricultural and Food Chemistry*, 47(11), 4638–4644. <https://doi.org/10.1021/jf990266t>
- Katz, E. E., & Labuza, T. P. (1981). Effect of water activity on the sensory crispness and mechanical deformation of snack food products. *Journal of Food Science*, 46(2), 403–409. <https://doi.org/10.1111/j.1365-2621.1981.tb04871.x>
- Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J., & Holčíková, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and Analysis*, 19(4), 252–276. <https://doi.org/10.1016/j.jfca.2005.04.014>
- Meléndez-Martínez, A. J., Vicario, I. M. & Heredia, F. J. (2007). Review: Analysis of carotenoids in orange juice. *Journal of Food Composition and Analysis*, 20(7), 638–649. <https://doi.org/10.1016/j.jfca.2007.04.006>
- Miranda, G., Berna, A., González, R., & Mulet, A. (2014). The storage of dried apricots: the effect of packaging and temperature on the changes of texture and moisture. *Journal of Food Processing and Preservation*, 38(1), 565–572. <https://doi.org/10.1111/jfpp.12004>

- Mondelez International (2019). *Introducing the state of Snacking. Global consumer snacking trends study*. https://www.stateofsnacking.com/wp-content/uploads/2019/11/2019_MDLZ_stateofsnacking_report_GLOBAL_EN.pdf
- Moraga, G., Igual, M., García-Martínez, E., Mosquera, L. H., & Martínez-Navarrete, N. (2012). Effect of relative humidity and storage time on the bioactive compounds and functional properties of grapefruit powder. *Journal of Food Engineering*, 112(3), 191–199. <https://doi.org/10.1016/j.jfoodeng.2012.04.002>
- Mosquera, L. H., Moraga, G., de Córdoba, P.F., & Martínez-Navarrete, N. (2011). Water content-water activity-glass transition temperature relationships of spray-dried Borojó as related to changes in color and mechanical properties. *Food Biophysics*, 6(3), 397–406. <https://doi.org/10.1007/s11483-011-9215-2>
- Muralikrishna, M., Nanjundaswamy, A. M., & Siddappa, G. S. (1969). Guava powder preparation, packaging and storage studies. *Journal of Food Science and Technology*, 6, 93-8.
- Nowak, D., Gośliński, M., Wojtowicz, E. & Przygoński, K. (2018). Antioxidant properties and phenolic compounds of vitamin C-rich juices. *Journal of Food Science*, 83(8), 2237–2246. <https://doi.org/10.1111/1750-3841.14284>
- Okos, M. R. (1986). *Physical and Chemical Properties of Food*. American Society of Agricultural Engineers.
- Roig, M. G., Bello, J. F., Rivera, Z. S., & Kennedy, J. F. (1999). Studies on the occurrence of non-enzymatic browning during storage of citrus juice. *Food Research International*, 32(9), 609–619. [https://doi.org/10.1016/S0963-9969\(99\)00128-3](https://doi.org/10.1016/S0963-9969(99)00128-3)
- Roohbakhsh, A., Parhiz, H., Soltani, F., Rezaee, R. & Iranshahi, M. (2015). Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. *Life Sciences*, 124, 64–74. <https://doi.org/10.1016/j.lfs.2014.12.030>
- Singleton, V. N., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, 299(1995), 152-178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Selvendran, R. R., & Ryden, P. (1990). Methods in Plant Biochemistry. In: P.M. Dey (Ed.), *Carbohydrates* (pp. 549). Academic Press.
- Silva-Espinoza, M. A., Ayed, C., Foster, T., Camacho, M. M., & Martínez-Navarrete, N. (2020a). The impact of freeze-drying conditions on the physico-chemical properties and bioactive compounds of a freeze-dried orange puree. *Foods*, 9(1). <https://doi.org/10.3390/foods9010032>
- Silva-Espinoza, M. A., Camacho, M. M., & Martínez-Navarrete, N. (2020b). Use of different biopolymers as carriers for purposes of obtaining a freeze-dried orange snack. *LWT - Food Science and Technology*, 109415. <https://doi.org/10.1016/j.lwt.2020.109415>
- Silva-Espinoza, M. A. (2021). *Design of the orange puree formulation and selection of the freeze-drying variables to obtain an orange snack* [unpublished Ph.D. Thesis, Universidad Politécnica de València].
- Syamila, M., Gedi, M. A., Briars, R., Ayed, C., & Gray, D. A. (2019). Effect of temperature, oxygen and light on the degradation of β -carotene, lutein and α -tocopherol in spray-dried spinach juice powder during storage. *Food Chemistry*, 284, 188-197. <https://doi.org/10.1016/j.foodchem.2019.01.055>

- Tang, Y. C., & Chen, B. H. (2000). Pigment change of freeze-dried carotenoid powder during storage. *Food Chemistry*, 69(1), 11–17. [https://doi.org/10.1016/S0308-8146\(99\)00216-2](https://doi.org/10.1016/S0308-8146(99)00216-2)
- Telis, V. R. N., & Martínez-Navarrete, N. (2009). Collapse and color changes in grapefruit juice powder as affected by water activity, glass transition, and addition of carbohydrate polymers. *Food Biophysics*, 4(2), 83–93. <https://doi.org/10.1007/s11483-009-9104-0>
- Telis, V. R. N., & Martínez-Navarrete, N. (2012). Biopolymers used as drying aids in spray- drying and freeze-drying of fruit juices and pulps. In: V.R.N. Telis (Ed.), *Biopolymer engineering in food processing* (pp. 279–325). CRC Press. <https://doi.org/10.1201/b12048>
- Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y., & Shi, J. (2008). Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chemistry*, 106, 545-551. <https://doi.org/10.1016/j.foodchem.2007.06.046>
- Zou, Z., Xi, W., Hu, Y., Nie, C. & Zhou, Z. (2016). Antioxidant activity of Citrus fruits. *Food Chemistry*, 196, 885–896. <https://doi.org/10.1016/j.foodchem.2015.09.072>