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Impact of freeze-drying shelf temperature on the bioactive compounds, physical properties and sensory evaluation of an orange product

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Abstract

Freeze-drying is a good alternative means of obtaining fruit products, with a significant amount of thermolabile bioactive compounds. Despite the excellent benefits this method provides the dehydrated product, its main drawback is its high cost due to the duration of the process. Heat may be applied to the shelf so as to shorten the process time, as long as this does not affect the quality of the product. In this study, the impact of the freeze-drying shelf temperature, 30 and 50 ºC, on the bioactive compounds of the product obtained from an orange juice formulated with gum Arabic and bamboo fiber has been considered, as has the temperature's effect on the porosity, color and mechanical properties of the cake and on the flowability and the rehydration capacity of the powder, together with the sensory evaluation of the rehydrated product. The results obtained point to 50 ºC as being the recommended temperature for the freeze-drying of this product. This temperature shortens the process time by 64%, promotes the vitamin C content with no effect on the total phenols and carotenoids, leads to the cakes having better mechanical properties and does not affect the flowability and the rehydration behavior of the powdered product.

Keywords

Vitamin C; total phenols; total carotenoids; antioxidant capacity; crispness; powder flowability**.**

1. Introduction

Fruits are foods of great importance in terms of human nutrition. In this group, citrus fruits are of particular importance, characterized by their distinctive flavor and as a good source of carbohydrates, dietary fiber, vitamins, minerals and biologically active phytochemicals, such as carotenoids and flavonoids, which provide antioxidant benefits (Hoffmann *et al.* 2003). The ingestion of these nutrients is closely related to the prevention of chronic diseases, some types of cancer, cardiovascular problems and brain disorders, such as Alzheimer's, so their consumption has a great and favorable impact on health (Boeing *et al.*, 2012) . However, the intake of these products is greatly limited by their availability and short shelf life. Dehydrated fruit, included fruit powder, may be a good alternative to lengthen the product's shelf life, because its low water activity will prevent chemical and enzymatic reactions that will deteriorate it. Consumption in dehydrated form or the subsequent rehydration of the powder would permit fruit intake in a comfortable, safe and healthy way.

For many years, hot air has been the most commonly used food dehydration method. However, its main problem is related to the loss of thermolabile compounds and sensory properties as a consequence of the heat treatment. Freeze-drying is a method characterized by the fact that it offers high quality products, based on sublimating a frozen sample at low pressures, without exposing the product to high temperatures (Que *et al.*, 2008). Of the benefits of freeze-drying, the preservation of the taste, flavor and thermo-sensitive compounds with biological activity, together

with the great rehydration capacity of the obtained products (Serna Cock *et al.*, 2015), are of special importance in the case of fruit.

Despite the excellent benefits this method provides the dehydrated product, its main drawback is its high cost due to the long duration of the process, which has limited its application being extended to the food industry. In recent years, nevertheless, the growing consume interest in healthy nutrition has led to an emerging interest in studying the optimization of the process variables trying to find alternatives to the reduction of time and, in turn, the energy cost, without sacrificing the quality of the product (Salazar *et al.*, 2018) (Ceballos *et al.*, 2012) (Babić *et al.*, 2009). Of the process variables, the shelf temperature seems to be the one with the greatest impact on process time, although it may also be the one that most compromises the properties of the product (Shishehgarha *et al.*, 2002).

Based on the above, the objective of this study was to evaluate the effect of the freeze-drying shelf temperature on the properties of the cake and powder obtained from an orange juice formulated with gum Arabic and bamboo fiber. The analyses carried out were related to the retention of bioactive compounds, porosity and the mechanical, color and flow properties, and the powder behavior against rehydration included the sensory properties of the rehydrated juice.

2. Materials and methods

2.1. Raw material and formulation

Orange (*Citrus x sinensis* var. Navelina), always acquired in the same chain of supermarkets in Valencia, was used for the study. To obtain the juice, a domestic juicer was used (Braun MPZ 6, Spain). To the juice (12° Brix and 88 g of water / 100 g juice) gum Arabic (GA, Scharlau, SL, and Spain) and bamboo fiber (BF, Vitacel, Rosenberg, Germany) were added in a ratio of 100:5:1, respectively (Agudelo *et al.*, 2017), and homogenized with a processor (Thermomix TM 21, Vorwek, Spain) at 2500 rpm for 40 s and subsequently at 9200 rpm for 40 s. The formulated juice (FJ) presented 82 g of water / 100 g and 18 °Brix.

2.2. Freeze-drying

The orange juice was placed on aluminum plates, of 25 cm in diameter and 1 cm thick, and frozen (Liebherr Medline, LCT 2325, Austria) at -45°C for 48 h. The drying step was carried out using a Telstar Lyo Quest 55 (Spain) freeze-dryer operating at -50 ºC in the condenser, p=0.05 mbar and changing the shelf temperatures between 30 and 50 °C for 48 or 18 h. The selection of these times, after which both samples reached around 4% water content, was based on previous experiments (data not shown).

2.3. Analytical determinations

The formulated orange juice and all the freeze-dried samples were analyzed as to the water content, vitamin C content (VC), antioxidant activity (AOA), total phenolics (TP) and carotenoids (TC). The freeze-dried cakes obtained from the orange juice were coded as C (30°C) and C (50°C), depending on the freeze-drying shelf temperature, and the mechanical properties, color and bulk density of the freeze-

dried cakes were also analyzed. In addition, the freeze-dried cakes were crushed in a food processor (Thermomix TM 21, Vorwek, Spain), at 3700 rpm for 20 s. The crushed cakes, in batches of about 40 g, were sieved through an 800 μm mesh (CISA, Spain). A vibrating drum (AMP0.40, CISA, Spain), 50 Hz, for 5 minutes was used to this end. The powders (particle size <800 μm) obtained from C(30°C) and C(50°C), were called P(30°C) and P(50°C), respectively and were characterized as to the particle size distribution, angle of repose, color, porosity and Hausner and Carr indexes. The powder rehydration behavior and the sensory properties of the rehydrated product were also determined.

2.3.1. Water content

The water content of the freeze-dried samples was analyzed by coulometric Karl-Fischer titration (C10S Compact, Mettler Toledo, USA).

2.3.2. Vitamin C

To determine the VC, dehydroascorbic acid (DHAA) was reduced to ascorbic acid (AA) using DL-dithriothreitol (Sánchez-Mata *et al.*, 2000) (Sánchez Moreno *et al.*, 2003) (Xu *et al.*, 2008) (Xu and Chang, 2007).The analyses were performed by high performance liquid chromatography (HPLC) (Jasco, Cremella, Italy), using a UV-visible diode array detector (MD-1510). The HPLC conditions were: Kromaphase 100-C18, 5 mm (4.6 x 250 mm) column (Scharlab SL), mobile phase oxalic acid (0.1%), 20 μL of volume injected; flow rate 1ml / min, detection at 243 nm at 25 ° C. The vitamin C was identified by its retention time and quantified by integrating the

peak areas, using ascorbic acid (Panreac, Spain) as a standard. In order to make the results of the samples before and after being freeze-dried comparable, the results were expressed in dry basis.

2.3.3. Total phenolics

The determination of TP was carried out using the Folin-Ciocalteu method (Benzie and Strain, 1999), which is based on the fact that the phenolic compounds react with the Folin-Ciocalteu reagent, at basic pH, turning blue , which can be determined spectrophotometrically. The absorbance at 765 nm was measured with the same UV-visible spectrophotometer previously described. To quantify the total phenols, solutions of different concentrations of gallic acid (Sigma-Aldrich) were prepared and used as a standard curve. The results were expressed as mg GAE / 100 g dry solutes.

2.3.4. Total carotenoids

The determination of TC was carried out by spectrophotometry (AOAC, 1996). For the extraction, 0.25 g of fresh fruit or 0.15 g of powder were taken, and 9 mL of a mixture of hexane:acetone:ethanol (50:25:25) was added. After stirring for 30 min in the dark, the sample was centrifuged (GYROZEN 123GR) at 10000 rpm and at 4 °C for 10 minutes. Subsequently, the supernatant was taken and for each mL obtained, 10 µL of distilled water was added and stirred for 2 min. The absorbance was measured at a wavelength of 446 nm in the previously mentioned UV-visible spectrophotometer. For quantification purposes, a calibration curve of β-carotene

(Fluka-Biochemika, USA) was used as standard. The analyses were performed in triplicate and the results were expressed as mg β-carotene / 100 g dry solute.

2.3.5. Antioxidant activity

The antioxidant activity (AOA) of the samples was evaluated using the DPPH and FRAP methods. The first method is based on the ability of antioxidant substances to capture free radicals (Puupponen-Pimiä, 2003). 1, 1-Diphenyl-1-picrihydrazil (DPPH), is a stable free-radical that is purple in color. As a result of the donation of an electron or a proton by a compound with antioxidant activity, the coloration disappears. The absorbance at 515 nm was measured in an UV-visible spectrophotometer (V-1200, VWR, International EuroLab S.L., Spain). The results were expressed in% DPPH according to Eq. (1).

$$\%DPPH = \frac{A_{control} - A_{sample}}{A_{control}} \text{ x } 100 \ldots\ldots\ldots\ldots \textit{(1)}$$

Where:

$A_{control}$= absorbance of the control (absorbance of the sample at time 0);

A_{sample}= absorbance of the sample when the reaction has stabilized.

The principle of the second method, FRAP (antioxidant power of ferric reduction), is based on the increase in absorbance due to the formation of the 2, 4, 6 trypyridyl-s-triazine (TPTZ) -Fe (II) complex in the presence of a reducing agent. For the ferric reducing ability of samples, the FRAP analysis was used. Absorbance values were taken using the same spectrophotometer described above at 593 nm.

In both cases, the results were expressed as mmol of trolox equivalent (TE) / 100 g dry solutes. Solutions of different concentrations of trolox (Sigma-Aldrich, Germany) were prepared and used as a calibration curve.

2.3.6. Mechanical properties

To evaluate the mechanical properties of the freeze-dried cakes, a puncture test was carried out using a texturometer (TA-TXT2i, Stable Micro Systems, Ltd. Godalming, UK) with a cylindrical probe of 10 mm in diameter. Samples previously cut into a circular shape by using a punch, 2.30 cm in diameter, were penetrated a fixed distance of 5 mm at a constant rate of 2.00 mm/s. The force-distance curve was recorded. Seven replicates were carried out.

The mechanical properties of powdered products were analyzed following the methodology proposed (Telis and Martinez-Navarrete, 2010). A compression test was performed using the same texturometer. The powder was deposited in aluminum containers of 11.5 mm in diameter and 4.5 mm in height and were compressed with a cylindrical punch of 10 mm in diameter and a fixed distance of 3 mm at a constant rate of 0.05 mm/s. The force-distance curve was recorded and the maximum force related to the sample mass was the parameter selected.

2.3.7. Color measurement

A portable spectrophotometer (CM2600D, Konica Minolta Sesing, INC., Japan) was used, using the 5mm diameter measurement window, taking illuminant D65 and observer 10°as system reference. CIEL*a*b coordinates were obtained and the angle hue (h*) and chroma (C*) were calculated by using equations (2) and (3),

respectively. The color of the freeze-dried cakes was measured on samples previously cut into a circular shape by using a punch of 2.30 cm in diameter. To avoid the influence that the different degree of compaction may have on the color of the powdered products, this was measured after being subjected to the mechanical compression test (2.3.6). The procedure proposed (Telis and Martinez-Navarrete, 2010) was followed to this end. Briefly, after the compression test, the capsule with the sample was quickly inverted and forced against the optical glass placed on the spectrophotometer window, which was the same described for cake color measurements (section 2.3). Measurements were taken in triplicate for each sample.

$$h^* = arctg\left(\frac{b^*}{a^*}\right) \qquad \ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$C^* = \left(a^{*2} + b^{*2}\right)^{0,5} \qquad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

2.3.8. Bulk density

The bulk density of a solid food is the ratio between the weight and volume of a portion, including the voids and pores it contains (Eq. 4). The size of freeze-dried cakes previously cut into a circular shape by using a punch of 2.30 cm in diameter was precisely weighed (Mettler Toledo, XS204DR, Switzerland) and measured with a Vernier caliper.

$$\rho_b = \frac{m}{V} \ldots\ldots\ldots \quad (4)$$

Where:

ρ_b = bulk density (g cm^{-3})

m = mass (g)

V = volume = $\pi r^2 h$, r and h being the exact radius and height of the weighted piece (cm^3).

2.3.9. Particle size distribution

A batch of about 40 g of the obtained powders, with a particle size of under 800 μm, precisely weighed, was sieved in descending order of mesh sizes of 500, 300, 200, 150 and 100 μm. The same vibrating drum and conditions previously described were used. The powder collected in each sieve and in the bottom was weighed. The relative sample mass retained in each sieve (Eq. 5) was used to find out the particle size distribution. The mean particle size was also calculated (Eq. 6). The procedure was carried out in triplicate.

$$F_i = \frac{m_i * 100}{m} \quad \ldots\ldots (5)$$

$$MPS = \frac{\sum_i (m_i \emptyset_i)}{m} \quad \ldots\ldots (6)$$

F_i= relative frequency retained in each sieve (%), m_i= powder mass retained in each sieve (g); m= total sieved powder mass (g); MPS: mean particle size (mm); Θ_i= each sieve mesh (mm).

2.3.10. Angle of repose

The objective of this analysis is to evaluate the powder flowability. This is the angle formed by the slope of the mountain created by the powder when dropped onto a horizontal surface and the surface itself. The test was carried by pouring 15 g of the powdered product into a funnel (top diameter= 80 mm, stem diameter = 11 mm, stem length = 29 mm, overall height = 85 mm), the base placed at a height of 5 cm from

the horizontal. The diameters of at least 3 different points and the maximum height of the product cone formed were carefully measured with a Vernier caliper and Eq. (7) was applied to calculate the angle of repose (α^o) (Gallo *et al.*, 2011), taken as the mean value of 3 replicates.

$$\alpha^\circ = arctan* (\frac{2h}{d}) \text{ …….. } (7)$$

Where h = height from the top of the product cone formed (cm); d = mean cone product diameter (cm).

2.3.11. Porosity and the Hausner and Carr indexes

The porosity (ε) of the powder was calculated from the true and tapped densities (Eq. 8). The true density (ρ) of each sample was calculated from its individual components (Eq. 9). In this case, the water content (previously analyzed) and carbohydrates (by difference) were considered to be the main components of the samples. The tapped density (ρ_t) was calculated by dropping approximately 10 mL of the corresponding powder into a graduated tube, with the help of a funnel. The weight and volume ratio of this sample was called the apparent density (ρ_a). The previous sample was compacted using a vortex (1200 rpm, 10 s) and the weight and volume were recorded to calculate ρ_t.

$$\varepsilon = \frac{\rho - \rho_t}{\rho} \text{ …… } (8)$$

$$\frac{1}{\rho} = \frac{x_w}{\rho_w} + \frac{x_{CH}}{\rho_{CH}} \text{ …… } (9)$$

Where ε is the porosity; ρ, and ρ_t are the true and tapped densities, respectively; x and ρ_i are the mass fraction and density, respectively, of the water (i=w) and

carbohydrates (i=CH) of the mixture, with ρ_w (20 ºC) 0.9976 g/cm^3 and ρ_{CH} (20 ºC) 1.4246 g/cm^3 (Choi and Okos, 1986).

The Hausner index (Eq. 10) is a value that is also related to the powder flowability while the Carr index (Eq. 11) refers to the ability of powdered substances to be compacted.

$$I_H = \frac{\rho_t}{\rho_a} \text{..... } (10)$$

$$I_C = 100\frac{\rho_t - \rho_a}{\rho_t} \text{..... } (11)$$

Where:

I_H= Hausner index; I_C = Carr index (%); ρ_t= tapped density (g/ml); ρ_a= apparent density (g/ml)

2.3.12. Rehydration behavior

The powder wettability, the color and the flow behavior of the rehydrated powders were selected for the purposes of characterizing the rehydration behavior of the powders.

A modified standard method for milk powder (UNE 34849, 1986), was used to determine the wettability. Briefly, 10 g of the powder were weighed and dropped progressively (for 25 s) into 250 g of water, measuring the time (s) from when the powder dropped until all the particles were wetted.

For the rehydration, the powder with a particle size of <100 μm, obtained from the sieving carried out to characterize the particle size distribution (section 2.4.1), was

used. The samples were rehydrated to reach exactly the same water content as that of the formulated juice before being freeze-dried. Eq. (12) and (13) were used to calculate the ratio of powder:water to be mixed. The rehydration was performed under controlled stirring (800 rpm for 10 minutes every 45 g) and at 20 ºC. Once the powder was rehydrated, it was left to rest for 24 h at 4 °C, before its subsequent rheological test and color measurement. The rehydrated samples obtained from P(30°C) and P(50°C), were coded as R(30°C) and R(50°C) respectively. The rehydration of each sample was carried out in triplicate.

$$m^{rh} = m_p + m_w \text{ ……. } (12)$$

$$m_p * (1 - x_w^p) = m^{rh}(1 - x_w^{rh}) \text{ ……. } (13)$$

Where x_w^p is the water content of the powder; x_w^{rh} is the desired water content of the rehydrated product; m^{rh} the mass of rehydrated product to be obtained (45 g); m_p is the mass of the powder; m_w is the mass of water to be added.

The flow behavior was characterized using a controlled stress rheometer (KINEXUS KNX2210, Malvern, UK) with cylinder geometry (C25 SW1569SS). The shear stress was recorded by applying a shear rate sweep from 0 to 150 s^{-1} at 8 °C.

To measure the color of the rehydrated juice, buckets 4 cm high and 1 cm thick were used. The physical color was measured from the reflection spectra, which was obtained on a black background. The same spectrophotometer described above was used and the h* and C* were also calculated (Eq. 2 and 3).

2.3.13. Sensory analysis

A triangular discriminatory test was performed with a panel of 30 tasters in order to determine whether there are significant differences between the two samples of orange juice, R (30°C) and R (50°C). Two triads were prepared per taster, where each triad had a duplicate and a different sample. Each sample was coded with three random digits and was presented an equal number of times in each of the possible positions: BAA, AAB, ABA, ABB, BBA, BAB in random order following a Williams design. Each taster evaluated the samples of two triads in the indicated order and marked the sample that they considered different. Additionally, tasters were told to indicate the sample that they liked the most.

2.4. Statistical analysis

Several analyses of variance (ANOVA) were carried out to evaluate the effect of temperature on the properties of freeze-dried products. The method used to discriminate between means was Fisher´s least significant difference (LSD) procedure. When $p<0.05$, significant differences were assumed between the samples.

3. Results and Discussion

The freeze-dried samples did not show a significant difference ($p<0.05$) as regards the water content, the obtained values being 0.039±0.005 and 0.041±0.005 g water/g sample when the drying was carried out at 30 and 50 ºC, respectively. In both experiments, a freeze-dried product with approximately the expected 4 g water/100 g sample was obtained.

As reported in Table 1, both TP and TC decreased due as a result of freeze-drying, with no observed effect of the drying temperature. The most sensitive appear to be carotenoids. In the case of VC (Table 1), an increase was observed after freeze drying: the higher the drying temperature, the bigger the change ($p<0.05$). This may be due both to the effect of temperature on the inactivation of the ascorbate peroxidase enzyme, which uses vitamin C as a cofactor in its metabolic processes (Cuastumal Canacuan *et al.*, 2016), and also to the fact that the freeze drying process was shorter at a higher temperature: 18 h at 50 ºC vs. 48 h at 30 ºC. These changes caused a decrease in the antioxidant activity without, in general, there being any observed effect of temperature.

3.1. Characterization of the cakes

The cakes did not show any significant differences ($p<0.05$) as regards their bulk density (Table 2), which would indicate no influence of the drying temperature in terms of the porosity achieved by the sample.

As to color (Table 2), and as expected for this product, all the samples were found in the red-yellow quadrant. Although the samples showed no significant differences ($p<0.05$) in terms of luminosity, the cakes obtained at a higher temperature showed a slightly yellowish and less pure color. The calculated color difference between the two samples (Eq. 14) was in the order of 16.66 units, indicating that they are differences perceptible to the human eye (Bodart *et al.*, 2008).

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \ldots\ldots\ldots \textit{(14)}$$

An example of the graphs obtained from the mechanical test for each freeze-dried cake at both temperatures is shown in Figure 1. This profile gives us the evolution of the force as the punch of the equipment that penetrates the sample until a cutting effect occurs that coincides with its perimeter (10 mm). The multiple force peaks observed correspond to small fractures in the samples related to their crispiness while the continuous increase in the force corresponds to the progressive compaction. In both samples, the increase in F was of the same order, until reaching a maximum F value that was related to a significant break in the sample. This maximum force was higher ($p>0.05$) in the samples freeze -dried at 50 ºC (Table 2), indicating that these samples are less fragile

3.2. Characterization of the powdered products

To check whether the freeze-drying temperature affects the mechanical strength of the freeze-dried product, its particle size distribution was characterized after being crushed. In both cases, the mode of this distribution was found in the range of 0.3-0.5 mm and the median, corresponding to the particle size that leaves 50 % of the powder above and below it, was in the range of 0.15-0.20 mm (Fig 2).

As can be seen in Figure 2, the biggest differences between the two samples were observed for particle sizes of less than 0.2 mm, so that P(30°C) had more particles of between 0.10 and 0.20 mm and fewer of <0.1 mm, linked to an increased resistance to crushing in this case. However, when the mean particle size was calculated (Table 3), the samples were observed to exhibit no significant differences ($p >0.05$). The greater number of the smallest particles observed in P(50 °C) could

be related to a greater ease of attrition during the sieving applied for this analysis, when the samples were dried at a higher temperature. In this sense, the less fragile cakes obtained at 50 ºC would lead to harder particles after being crushed, which generate more fine powder during processing.

The angle of repose is a measurement of the powder flowability that is related to the cohesion between molecules, the chemical composition of the material, the size and shape of particles and the water content. As a reference, the angle of repose of some flours are in an interval from 30 to 50 (Swarbrick, 1997), in the same order as our results (Table 3), with no significant differences observed between the samples ($p>0.05$). According to the values obtained, the Spanish Royal Pharmacopoeia allows us to classify our products with an excellent flowability (Agencia Española de Medicamentos y Productos Sanitarios AEMPS, 2015), which is a favourable characteristic for a powdered product. The Hausner and Carr indexes, also related with the powder flowability, pointed to no significant differences between the samples ($p<0.05$). The drying temperature affected neither the color nor the mechanical properties of the powdered products (Table 3).

The calculated real density of these samples was 1.4246 g cm^{-3}. The apparent and tapped densities were 0.315 ± 0.003 and 0.394±0.004 for P(30ºC) and 0.367±0.016 and 0.455±0.014 for P(50ºC), respectively. From these data, the samples showed significant differences as regards porosity ($p<0.05$), of which there was less in sample P(50 °C), related to the fact that it is less fragile and when crushed, this sample generates more regular and rounded particles that would allow for a better packaging and, therefore, a lower volume of air. In addition, the greater presence in

this sample of fine particles (Fig. 2), capable of occupying the gaps between the larger particles, contributes in the same way.

3.3. Behavior of the powder against rehydration

In addition with particle size, the wetting time is mainly associated with the composition of the sample, especially its water content, so that the wetting time lengthens when the water content decreases (Ceballos Penaloza, 2008). In Table 4, it can be observed that there were no significant differences between the two samples ($p<0.05$), which was to be expected since there were no compositional differences between the samples.

The flow curves of the rehydrated powdered products were obtained, following the procedure described in section 2.3.12. The data obtained were fitted to the Ostwald-de Waele model (Table 4), where we can confirm the pseudoplastic nature of both products ($n<1$). From the values of the model parameters K and n, the viscosity at a shear rate of 100 s^{-1} was calculated (Table 4). There were no significant differences between the samples in any of the rheological parameters evaluated ($p>0.05$).

Rehydrated samples exhibited significant differences ($p<0.05$) in terms of luminosity and hue angle (Table 4), so that R(50ºC) was a more luminous and yellowish product with respect to R(30ºC). However, their overall calculated color difference (Eq. 15) gave a value of 1.2, which indicates that the color difference was not perceptible to the human eye (Bodart *et al.*, 2008).

3.4. Sensory analysis

To analyze the data of the triangular test, the correct answers were considered and the result was compared with the data corresponding to the statistical table of triangular tests to evaluate whether there is a significant difference between the samples at 95% probability.

From the total of 60 triangles evaluated, 20 correct answers were obtained. As the tabulated value (27) is greater than the experimental (20), it can be concluded that no significant differences were found between the two samples of rehydrated powder, R(30°C) and R(50°C), since the tabulated value represents the minimum number of correct answers required to find significant differences between two samples. After analyzing the results to the question of which of the samples the taster preferred, it was found that of the correct answers, 55% of the tasters chose sample R(30°C), 40% of the tasters R(50°C) and 5% liked the two samples equally; however, after the triangular tasting it has been seen that there are no differences between the samples.

4. Conclusion

As regards the bioactive compounds, to apply 50 ºC along drying doesn't affects TP nor TC and promotes an increase in VC content. Despite the freeze-drying temperature did not affect the porosity, color and crispiness of the cakes, the sample obtained at 50 ºC was more resistant to breakage due to the application of small forces. This facilitates the handling of this product. On the other hand, when subjected to larger shear forces such as those needed to obtain a powder, more fine particles were obtained when the drying was done at a higher temperature, which decreases the interparticle porosity and allows for a better packaging. The average

particle size and color of the powder were also not affected by the drying temperature. Moreover, neither did it affect the wetting time during rehydration nor the rheology and sensory perception of the rehydrated product. In view of the above, taking into account that freeze-drying at 50 ºC means that the process time is 64% shorter compared to the process at 30 °C, with the consequent energy savings, and also the fact that there is a limited impact on the properties of the product, which are even somewhat better when the product is dried at 50C, this temperature could be recommended as a means of obtaining freeze-dried orange juice to be consumed either as a snack or as a powder to be rehydrated.

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Table 1. Mean values ± standard deviation of the total phenols (TP, mg GAE /100 g db), total carotenoids (TC, mg β-C /100 g db), Vitamin C (VC, mg/100 db) and antioxidant activity (mmolTE/100 g db) measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing / antioxidant power) methods for the different samples(*).

	FJ	P(30ºC)	P(50ºC)
DPPH	1.05 ± 0.5^{b}	1.09 ± 0.08^{b}	0.93 ± 0.03^{a}
FRAP	3 ± 1^{b}	2.3 ± 0.5^{a}	2.2 ± 0.1^{a}
TP	705 ± 54^{b}	420 ± 5^{a}	378 ± 37^{a}
TC	54 ± 6^{b}	8.1 ± 0.8^{a}	8.4 ± 0.4^{a}
VC	236 ± 5^{a}	252 ± 8^{b}	276 ± 19^{c}

(*)FJ, P(30°C) and P(50°C): formulated orange juice and the corresponding powders obtained by freeze drying at 30 and 50 °C, respectively. GAE: gallic acid equivalent, TE: Trolox equivalent, db: dry basis. The same superscript letter (a-c) in rows indicates the homogeneous groups established by the ANOVA ($p<0.05$).

Table 2. Mean values (±) standard deviation of the different properties analysed for the cakes obtained from the orange juice freeze-dried at 30 ºC (C 30ºC) and 50 °C (C 50ºC).

Property(*)	P (30°C)	P (50°C)
ρ_b (g/mL)	0.13 ± 0.01[a]	0.13 ± 0.02[a]
L*	60.63 ± 3[a]	60.93 ± 5[a]
a*	12 ± 2[b]	8 ±2[a]
b*	41 ± 6[b]	34 ± 4[a]
C*	43 ± 6[b]	35 ± 4[a]
h*	74.3 ± 1.0[a]	77 ± 2[b]
F_{max} (N)	11 ± 3[a]	16 ± 5[b]

(*)ρ_b: bulk density; L*, C* and h*: luminosity, chroma and hue angle; a*, b*: color coordinates; $F_{máx}$: maximum force recorded in the mechanical test. The same superscript letter (a-b) in rows indicates the homogeneous groups established by the ANOVA ($p<0.05$).

Table 3. Mean values (±) standard deviation of the different properties analyzed for the powdered products obtained from the orange juice freeze-dried at 30 ºC (P 30ºC) and 50 °C (P 50ºC).

Property(*)	P (30°C)	P (50°C)
MPS (mm)	0.196 ± 0.009[a]	0.189 ± 0.015[a]
Angle of repose	30.9 ± 0.3[a]	29 ± 2[a]
Porosity (%)	77.5 ± 0.2 [b]	74 ± 1 [a]
I_H	1.25 ± 0[a]	1.240 ± 0[a]
I_C (%)	20 ± 3[a]	19 ± 3[a]
F_{max} (N/g)	404 ± 140[a]	415 ± 110[a]
L*	72 ± 1[a]	72 ± 1[a]
a*	14 ± 1[a]	13 ± 1[a]
b*	54 ± 1 [a]	54 ± 1 [a]
C*	56 ± 1[a]	56 ± 1[a]
h	75.1 ± 0.9[a]	75.6 ± 0.6[a]

(*)MPS: mean particle size; F_{max}: maximum force recorded in the mechanical test; I_H: Hausner index; I_C: Carr index; L*, C* and h*: luminosity, chroma and hue angle; a* and b*: chromatic color coordinates; The same superscript letter (a-b) in rows indicates the homogeneous groups established by the ANOVA ($p<0.05$).

Table 4. Mean values (±) standard deviation of the different properties analyzed for the rehydrated products obtained from the orange juice freeze-dried at 30 ºC (R 30ºC) and 50 °C (R 50ºC).

Property(*)	R (30°C)	R (50°C)
Wettability (s)	32 ± 2^{a}	30 ± 2^{a}
n	0.75 ± 0.03^{a}	0.753 ± 0.016^{a}
K (Pa s^{n})	0.05 ± 0.01^{a}	0.039 ± 0.003^{a}
Ƞ (mPa s)	10 ± 2^{a}	8.4 ± 0.6^{a}
L*	45.86 ± 0.10^{a}	46.65 ± 0.04^{b}
a*	3.9 ± 0.1^{b}	3.13 ± 0.06^{a}
b*	27 ± 1^{a}	26.85 ± 0.03^{a}
C*	27.0 ± 0.6^{a}	27.03 ± 0.04^{a}
h	81.66 ± 0.08^{a}	83.35 ± 0.12^{b}

(*)Parameters of the Ostwald de Waele model (K: consistency index; n: flow behavior index) and viscosity at shear rate= 100 s^{-1} (η). L*, C* and h*: luminosity, chroma and hue angle; a* and b* chromatic color coordinates; The same superscript letter (a-b) in rows indicates the homogeneous groups established by the ANOVA ($p<0.05$).

FIGURE CAPTIONS

Figure 1. Example of force-distance curves obtained from the puncture-compression test performed on the freeze-dried sample at 30ºC C(30ºC) and 50ºC C (50ºC).

Figure 2. Particle size distribution based on the percentage of weight of the powder retained in each sieve relative to the total sieved sample (Weight, %). Particle size intervals: the largest mesh of the sieves that the sample did not pass through - the smallest mesh of the sieves that the sample passed through. Mean values and standard deviation. P(30°C) and P(50°C): powdered products coming from the cakes obtained after the freeze-drying of the orange juice at 30 and 50 °C, respectively.

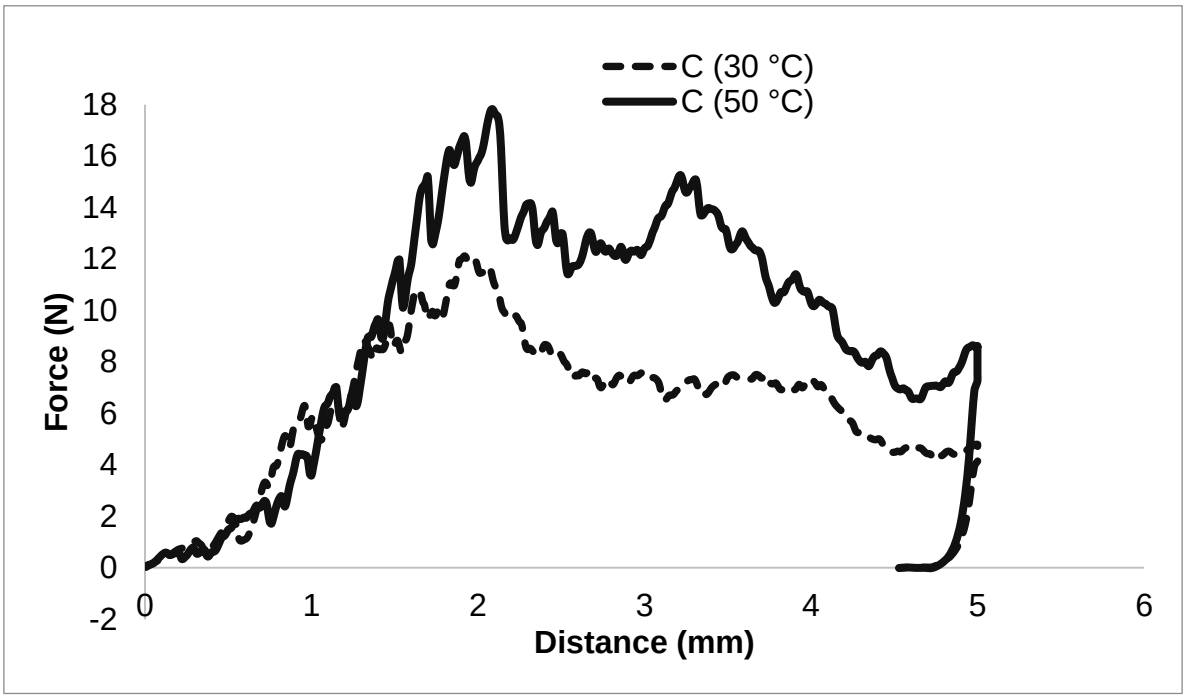

FIGURE 1

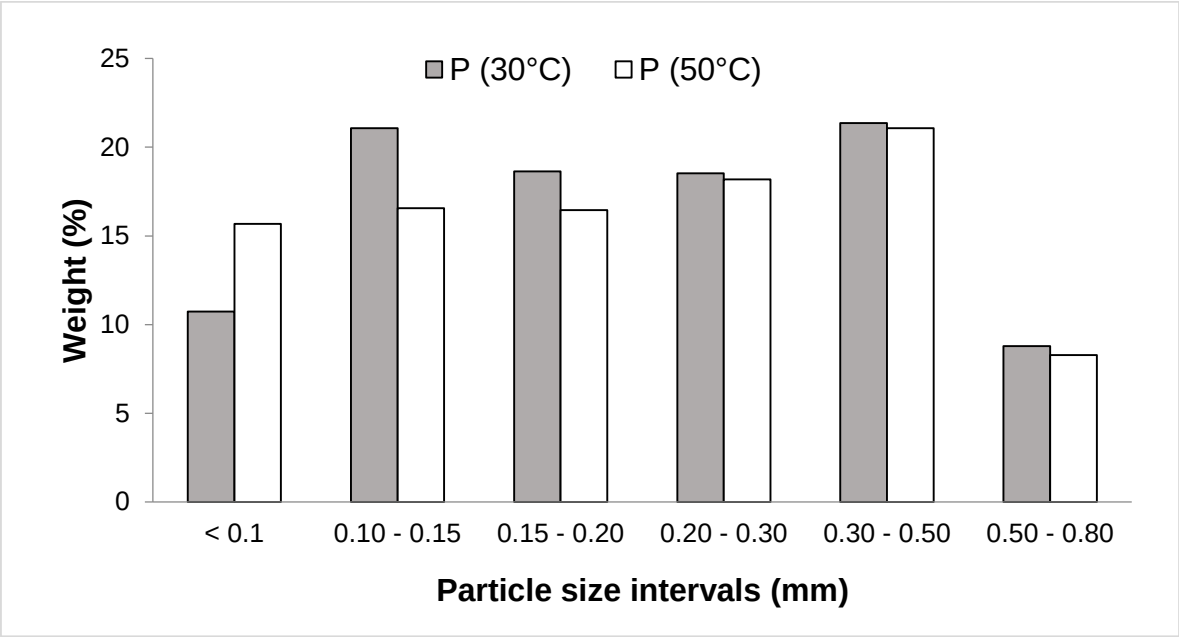
P (30°C)
P (50°C)
Weight (%)
25
20
15
10
5
0
< 0.1
0.10 - 0.15
0.15 - 0.20
0.20 - 0.30
0.30 - 0.50
0.50 - 0.80
Particle size intervals (mm)

FIGURE 2