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Development of dried functional foods: stabilization of orange pulp powder by addition of biopolymers

Short title: Stabilization of freeze-dried orange pulp by adding biopolymers

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ABSTRACT

The production of powdered food is an increasingly important industry due to the high stability and easy handling of those products. The aim of this work was to evaluate the effect of the addition of gum arabic in combination with bamboo fiber or cactus cladode mucilage on the physicochemical and antioxidant properties of orange pulp powder obtained by freeze drying. Additionally, the stability of the powders after 10 months of storage was evaluated. The following determinations were performed: moisture content and hygroscopicity, glass transition temperature, total phenolic and vitamin C content, antiradical and antioxidant capacities, compression test, color and FESEM analysis. The results showed that the inclusion of gum arabic in combination with bamboo fiber or cactus cladode mucilage to the orange puree previous to freeze drying improved their chemical and physical stability. The combination gum arabic- bamboo fiber resulted a better option than the system gum arabic-cactus cladode mucilage.

Keywords: bioactive, antioxidant capacity, fruit powder, freeze-drying, stability.

1. Introduction

Orange is one of the most important world fruit crops and is consumed mostly as fresh or juice because of its nutritional value and special flavor. Orange also contains large amounts of phytochemicals such as vitamin C and polyphenols that may act additively or synergistically to exert their antioxidant, anti-inflammatory, and anticancer effect, as well as their cardiovascular protection activities [1-3].

Freeze-dried orange powder may be a good alternative to retain the nutritional, functional and sensory properties of the fruit and to diversify its consumption possibilities [4]. This fruit powder can be used as ingredient to formulate foods or to reconstitute natural fruit juices. The

production of powdered food is an increasingly important industry due to the high stability and easy handling of those products. Nevertheless, the process used to obtain the powder should ensure the maximum quality of the product. In addition to the stability of the nutritional components, certain physical properties of the powder have to be considered. Food powders should appear homogeneous and keep free flowing properties during storage. In order to reduce stickiness, to inhibit caking, and to guarantee the safe handling and storage of food powders, the key factors to be managed are the strict control of moisture content and the storage at low temperatures. Moreover, to improve the quality and stability of the freeze-dried fruit, the addition of high-molecular weight additives to the product before drying as carrier and anticaking agents is a widely used alternative [5, 6]. The mechanisms by which these agents may contribute to the storage stability of hygroscopic powders are based on different factors that may be superimposed: competing with host powder components for moisture, acting as a physical barrier between particles -even without completely covering the powder surface-, increasing the glass transition temperature (T_g) of the amorphous phase, and forming a moisture protective barrier on the surface of otherwise hygroscopic particles [6].

Gum arabic (GA), is an edible biopolymer obtained as exudates of mature trees of Acacia. Chemically, GA is a complex mixture of macromolecules of different size and composition (mainly carbohydrates and proteins) [7]. GA has been widely used in the food industry as a stabilizer, thickener and/or an emulsifier agent.

Bamboo fiber (BF) is extracted from the plant *Bambusa vulgaris*. The chemical composition of bamboo has been studied, comprising mainly cellulose together with lignin and hemicellulose [8]. Some biologically active components in bamboo leaves and their potential health benefits have been widely studied. Many of these studies have revealed that bamboo leaf extract contains flavones glycosides, phenolic acids, coumarin lactones, anthraquinones

and amino acids. Thus, it has been correlated with multiple biological beneficial effects for health (anti-free radical, anti-oxidation, anti-aging, anti-fatigue, anti-bacteria and anti-virus properties) [9]. Its high molecular weight makes it a possible candidate to increase the Tg, with the added value of being a healthy vegetable fibre.

Opuntia ficus-indica, also known as cactus pear or nopal cactus, is a member of the plant family *Cactaceae* and the most commercially important cactus. It is a domesticated crop plant which can be found in arid and semiarid regions throughout the world. *Opuntia ficus-indica* cladodes, as its modified stems are called, have interesting biological activity, such as hypoglycemic, antibiotic, antimicrobial, antihypercholesterolemic, anti-inflammatory, and antioxidant activity [10]. These health benefits have been attributed to the cactus mucilage (CM) that can be extracted from the cladodes [11, 12]. CM has also been used as encapsulating agent, in combination with maltodextrin, in the microencapsulation of betalain rich extracts, showing excellent results and as good input of dietary fiber [5].

The aim of this work was to design new dried food products and to evaluate the performance of gum arabic in combination with bamboo fiber or cactus cladode mucilage as carrier agents in the freeze-drying of fresh orange pulp.

2. Materials and Methods

2.1. Materials

Oranges (*Citrus sinensis* var. Navel) were collected from two particular trees reserved for this study from a crop field located in Bétera (Valencia, Spain). GA was provided by Scharlab (Barcelona, Spain), while BF was purchased from Rettenmaier Ibérica (Barcelona, Spain). Cladode mucilage of *Opuntia ficus-indica* was extracted [5] from cladode medulla from a cultivar in Santiago del Estero (Argentina).

2.2. Freeze-drying process

Oranges were peeled, cut, and the pulp (O) was processed in a crushing machine (Thermomix TM 21, Vorwerk, Valencia, España). For obtaining orange pulp powders including carrier agents, the two mixtures of these last materials, gum arabic/bamboo fiber (GA-BF) and gum arabic/cladode mucilage (GA-CM) were added to O and the mixture was homogenized (Thermomix TM 21, Vorwerk, Valencia, España). The mass ratio of GA to O was 5% (w/w pulp) and the corresponding one to BF or CM was 1% (w/w pulp). Both mixtures and O were transferred to aluminium pans (6 cm diameter, 1 cm height), frozen (-40°C/ 48h) and freeze-dried (10^{-2} Pa/ -40°C/ 48 h, Telstar Lioalfa-6 Lyophyliser, Terrassa, Spain). The freeze-dried powders obtained: O-GB (orange pulp with GA-BF), O-GC (orange pulp with GA-CM) and OP (orange pulp powder) were stored in vacuum-sealed bags into desiccators over silica gel at room temperature. Powder characterization analyzes described below were carried out within the 48 h of being processed.

2.3. Characterization of powders

2.3.1. Moisture content and hygroscopicity

Moisture content (x_w) was determined gravimetrically placing accurately weighed samples of each powder (500 mg) in tared Petri dishes. Afterwards, they were vacuum dried in a vacuum oven (Vaciotem, J.P. Selecta, Barcelona, Spain) at 60 ± 1°C under a pressure of 100 mm Hg until constant weight. Hygroscopicity (H) assays were carried out according to Tonon et al. (2008) [13] with minor modifications. Samples (about 400 mg in tared Petri dishes) were placed at 25°C in an airtight plastic container comprising a NaCl (Scharlab SL, Barcelona, Spain) saturated solution (75.29% RH). After one week, each sample was weighed and H was expressed as g of water gained per 100 g of dry solids.

2.3.2. Glass transition temperature

Calorimetric analyses were carried out immediately after the dehydration process in order to determine the T_g by differential scanning calorimetry (DSC). About 10 mg of each sample

were placed into DSC pans (P/N SSC000C008, Seiko Instruments, Chiba, Japan), sealed and analyzed using a DSC 220CU-SSC5200 (Seiko instruments Inc., Chiba, Japan). The heating rate was 5ºC/min and temperature range scanned was -20 – 80ºC.

2.3.3. Total phenolic content (TPC)

The fruit extracts used for the quantification of total phenolics were prepared as follows: Powders were grounded in a crushing machine (Kenwood, CH580, Selingenstadt, Germany) and the fraction passing a 7 mm-sieve was extracted at room temperature with a methanol/water (70:30) mixture under magnetic stirring (400 rpm, Multistirrer Velp Scientifica, Usmate Velate, Italy), in darkness, for 30 min. The solid:solvent ratio used was 1:20 (w/v). Residues were separated by centrifugation at 5,867 × g and 4°C, for 10 min (Eppendorf centrifuge 5804 R, Wesseling-Berzdorf, Germany) and the supernatant was recovered and filtered (45 µm nylon filter). All extracts were obtained in triplicate. TPC of extracts was determined by the Folin–Ciocalteu colorimetric method [14]. Briefly, an aliquot of 250 µl extract was mixed with 15 ml water and 1.25 ml of Folin–Ciocalteu reagent (Sigma-Aldrich, Darmstadt, Germany). After 8 min, 3.75 ml of 7.5% anhydrous Na_2CO_3 (Scharlab SL, Barcelona, Spain) aqueous solution was added and absorbance was measured at 765 nm (UV-visible spectrophotometer Thermo Electron Corporation, MA, USA) after 2 h of incubation at room temperature in dark condition. As samples have a different composition as regards water and added carrier agents, results were referred to the orange's solutes (OS), in order to make the results comparable. In this sense, the TPC was expressed as g of gallic acid equivalents (GAE) per kg of OS (g GAE/kg OS). A standard curve ranging from 0 to 1000 ppm of gallic acid (Sigma-Aldrich, Schnelldorf, Germany) was used. Ascorbic acid exerts an interference to the response of the Folin-Ciocalteu assay [15], increasing the absorbance value read. Once the ascorbic acid content of the different samples was

149 established (detailed in section 2.3.4.), its contribution to the absorbance value obtained was
150 determined by means of a calibration curve, and the corresponding correction was done.
151 2.3.4. Vitamin C (VCC)
152 Total VCC was determined reducing dehydroascorbic acid to ascorbic acid [16], by high-
153 performance liquid chromatography (HPLC) (Jasco, Cremella, Italy). In brief, 0.5 g of
154 powder were mixed with 2 ml of a 20 g/l DL-dithiothreitol solution (Scharlab S.L.,
155 Barcelona, Spain) for 2 h at room temperature and in a dark condition. Afterwards, VCC was
156 extracted [17]. For that, 1 g of this mixture was extracted with 9 ml 0.1% oxalic acid
157 (Scharlab S.L, Barcelona, Spain) under stirring for 3 min and filtered through a 0.45 μm
158 membrane filter. Finally, the VCC was determined by HPLC with the following conditions:
159 Kromaphase 100-C18 column (4.6 x 250 mm, 5 mm) (Scharlab S.L, Barcelona, Spain);
160 mobile phase 0.1% oxalic acid, 20 ml volume injection, 1ml/min flow rate. Detection was
161 made at 25ºC and at 243 nm using a detector UV-visible MD-1510. A standard solution of L
162 (+) ascorbic acid (Scharlab S.L., Barcelona, Spain) in the range of 10-530 ppm was prepared.
163 The VCC was calculated as g of ascorbic acid (AA) per kg of OS (g AA/ kg OS).
164 2.3.5. Antiradical activity (ARA) and antioxidant activity (AOA) analyses
165 The ARA and AOA of the methanolic extract obtained for the quantification of TPC was
166 determined by the DPPH$^{\cdot}$ and FRAP methodologies, respectively.
167 2.3.5.1. DPPH$^{\cdot}$ scavenging capacity assay
168 The methodology by Brand-Williams et al. (1995) [18] was followed in order to determine
169 the ARA of the samples. A 30 μl aliquot of extract was placed in a cuvette containing 3 ml of
170 a DPPH$^{\cdot}$ (Scharlab S.L, Barcelona, Spain) methanolic solution (A_0 = 1.00 ± 0.02). After 6 h
171 of incubation in dark conditions, the absorbance at 517 nm was determined using a
172 spectrophotometer (Thermo Electron Corporation, MA, USA). The percentage of the
173 antiradical activity (ARA %) was calculated according to the following equation:

$$\text{ARA \%} = \left(1 - \frac{A_f}{A_0}\right) 100\ \% \quad (1)$$

where A_0 is the absorbance of the $DPPH^{\cdot}$ solution before addition of the antioxidant, whereas A_f denotes the final absorbance after 6 h.

The results were converted to mmol trolox equivalents (TE) per kg of OS (mmol TE/kg OS) using a Trolox (Sigma-Aldrich, Schnelldorf, Germany) calibration curve in the range 30-250 ppm.

2.3.5.2. FRAP assay

For the ferric reducing ability of samples, aiming to evaluate the AOA of samples, the FRAP assay was performed [19, 20]. The FRAP solution was prepared by mixing 2.5 ml 10mM TPTZ (2,4,6-tripyridyl-s-triazine, Sigma-Aldrich, Schnelldorf, Germany) solution (in 40mM HCl solution), 2.5 ml 20mM $FeCl_3 \cdot 6H_2O$ (Sigma-Aldrich, Schnelldorf, Germany) solution and 25 ml 0.3M buffer acetate (pH 3.6). 30 μl of powder extracts (obtained according to section 2.3.3.), 30 μl water and 900 μl of the FRAP solution (kept at 37ºC throughout the whole analysis) were mixed and allowed to react for 30 min at 37ºC in darkness. Absorbance of the colored product (ferrous tripyridyltriazine complex) was then taken at 593 nm. Results were expressed as mmol trolox equivalents (TE) per kg of OS (mmol TE/ kg OS), using a Trolox (Sigma-Aldrich, Schnelldorf, Germany) calibration curve in the range 0-250 ppm.

2.3.6. Compression test

A TA-XT Plus (Stable Micro Systems, Godalming, UK) texture analyzer was used to perform the mechanical compression test [6]. The aluminum sample holder (diameter: 11 mm, height: 5.5 mm) was hollowed at the bottom, where a polyethylene disc (diameter: 10 mm, thickness: 1 mm) was inserted. The sample holder remained static during the measurements, while the sample was compressed at a rate of 0.05 mm/s over a distance of 3 mm. The maximum force attained was denoted by F_{max}.

2.3.7. Color analysis

For color determination, the compressed samples were positioned over a reflectance glass (CR-A51, Minolta Camera, Tokio, Japan) fixed upon the spectrophotometer lens (mod. CM-2002, Minolta Camera, Tokio, Japan) and forcing them against the surface in order to obtain a uniform sample surface and thickness [6]. Color was measured using the CIEL*a*b* color coordinates (L* [black (0) to white (100)], a* [greenness (−) to redness (+)] and b* [blueness (−) to yellowness (+)]) with a D65 illuminant and 10° observer. Results are expressed in terms of the polar coordinates L*C*h*, being L* the same as above, C* the chroma or saturation index ($C^* = (a^{*2} + b^{*2})^{1/2}$) and h* the hue ($h^* = \text{arctg}\ (b^*/a^*)$). Additionally, the stored samples were characterized according to their total color change (ΔE_t) according to Eq. (2).

$$\Delta E_t = \sqrt{(L^* - L_0^*)^2 + (C^* - C_0^*)^2 + (h^* - h_0^*)^2} \quad (2)$$

2.3.8. FESEM analysis

A morphological analysis was performed by field emission scanning electron microscopy (FESEM) observations. FESEM images were acquired by a Zeiss Ultra 55 (Carl Zeiss NTS GmbH, Oberkochen, Germany) with an accelerating voltage of 2 kV and at a working distance of 5 mm. Samples surfaces were previously coated with a thin platinum layer in a High Vacuum Coater EM MED020 (Leica Microsystems, Wetzlar, Germany).

2.4. Storage stability test

All three powder samples (OP, O-GB, and O-GC) were stored under vacuum and in a dark condition for 10 months at room temperature (25ºC) in order to evaluate their stability during storage. The samples were subjected to the following characterization analysis: AOA, ARA, TPC, VCC, color and mechanical properties.

2.4. Statistical analysis

Data are expressed as means and standard deviation of six replicates (extractions in triplicate, extract measurements in duplicate) for chemical analyses and of triplicate for physical tests.

Analyses of variance (ANOVA) were carried out at a 0.05 significance level, in order to evaluate differences between samples. All statistical analyses were performed using Statgraphics Plus 5.1.

3. Results and discussion

3.1. Moisture content and hygroscopicity

Table 1 shows the results obtained from the x_w and H assays. Moisture content is an important powder property, which is related to the drying efficiency, powder flowability, stickiness, and storage stability due to its effect on glass transition and crystallization behavior [21]. Regarding mass fraction of water in samples, OP presented a slightly higher value yet statistically different ($p < 0.05$) than O-GB and O-GC. No significant difference was found between both solute-added systems ($p > 0.05$). Jafari et al. (2016) [22] obtained a value of 2.31% when stabilizing saffron petal's extract by freeze drying with a mixture of maltodextrin and GA.

H was found to be significantly different ($p < 0.05$) for all three samples. OP showed a clear higher value than those obtained for the solute-containing samples. H of powdered fruit extracts is related to the low molecular weight sugars and organic acids with low T_g and moisture content, which leads to a high H [23]. The addition of high molecular solutes to fruits favors the diminution of this phenomenon [24, 25]. Although both O-GB and O-GC samples presented significantly different H values, there is no practical difference between these values. In the open literature, a wide range of H values can be found for stabilized fruit material, due not only to the nature of the material, but also to the relative humidity level established for the analysis.

3.2. Glass transition temperature

The temperature which defines the transition of a material from the glassy to the rubbery state is called the T_g. This physical change, which results in a decrease in the material viscosity,

247 exerts an accelerating effect on the rate of certain degradative reactions which are controlled
248 by diffusion, such as Maillard and oxidation reactions [26]. Moreover, above T_g the material
249 tends to be more susceptible to physical undesirable changes, namely crystallization, collapse
250 and stickiness [23]. Also, crispy products are observed to undergo a loss of crunchiness,
251 becoming texturally unacceptable above T_g [27].
252 The T_g obtained for the three powder formulations are shown in Table 2. It can be observed
253 that the addition of the high-molecular weight carrier agents to the orange pulp increased the
254 T_g in approximately 13ºC. Several researchers have also confirmed this behavior when high
255 molecular weight solutes were added to fruit pulp or juice [6, 25, 28]. In this manner,
256 considering the midpoint of the glass transition, the T_g values for O-GB and O-GC samples
257 were above ambient temperature (20ºC), assuring certain degree of chemical stability against
258 degradative reactions.
259 3.3. Bioactive content, antiradical and antioxidant activity of samples
260 The TPC, VCC and antiradical ($DPPH^{\cdot}$) and antioxidant (FRAP) properties of fresh orange
261 pulp and freeze-dried orange powders, with and without carrier agents, before and after
262 storage are shown in Table 3. The losses in these four parameters with storage were also
263 quantified and reported in the same table.
264 Initial TPC of orange samples varied from 3.93 to 6.35 g GAE/kg OS. These results were in
265 agreement with those reported in other studies of citrus [29-31]. Since orange fruits have a
266 significant amount of ascorbic acid, total phenolics values were corrected by discounting the
267 ascorbic acid interference [15]. Corrected and non-corrected phenolic contents differed by
268 approximately 10%. Comparison with reported values in the open literature for orange
269 phenolic contents is sometimes difficult since many of them are not corrected, although they
270 were also determined by the Folin–Ciocalteu colorimetric method. Thus, these contents may
271 be in some cases overestimations of the real values. The comparison of O sample with all

three powders showed that freeze-drying process favored the extraction of phenolic compounds ($p < 0.05$), while no significant difference ($p > 0.05$) was found among TPC values of the powdered samples. It was observed an increase of up to 60% when compared to O sample. The increase in phenolic compounds due to the freeze-drying process has been observed in other studies [32, 33]. This increase could be explained because during the freezing step prior to freeze drying, ice crystals formed can break the remaining cellular structure of the fruit. This could facilitate the subsequent entry of the solvent and could consequently improve the extraction of the phenolic compounds. Moreover, the low processing temperatures used in freeze drying would preserve phenolic compounds from degradation. Also, the high porosity and low particle size of the powder samples increase the superficial area available for mass transfer, favors the surface of contact with the solvent and then causes an increased yield extraction. On the contrary, freeze drying process produced a significant ($p < 0.05$) loss of vitamin C in the case of OP (23.6%) and O-GC sample (17.0%). Nevertheless, the presence of GA in combination with FB protected this compound from degradation during this process, and no significant differences ($p > 0.05$) were observed between O and O-GB.

The antioxidant capacity of fruits is important for assessing their health promoting properties. In our study, the ARA and AOA of orange samples were evaluated using $DPPH^{\cdot}$ free radical-scavenging and FRAP assays, respectively. Despite the ARA was significantly ($p < 0.05$) higher only for O-GC sample, the AOA of all the freeze-dried samples was significantly ($p < 0.05$) higher than that of fresh fruit. This could be attributed to the increase in the phenolic content of the dried samples. Some recent studies have shown that freeze-dried plant materials contain higher concentration of antioxidants, such as polyphenolics, and hence, higher antioxidant activity as compared to fresh plant materials [33, 34]. As it has been

already said, the rupture of the vegetable tissue as a consequence of ice crystal formation during raw material freezing prior to freeze drying could favor the further extraction process.

After 10 months of storage in dark and vacuum condition, the powdered samples were analyzed in order to evaluate the changes in TPC, VCC, AOA and ARA that could have taken place during this period. The results are summarized in Table 3.

Despite no significant protective effect of solutes was observed against TPC and VCC during the freeze-drying of samples, a significant ($p < 0.05$) greater decrease in both compounds was suffered by OP sample after 10 months, compared to O-GB and O-GC, evidencing the protective role of the wall materials towards polyphenols and vitamin C during storage.

Analyzing the ARA and AOA of stored powdered samples, the three samples evidenced a significant diminution ($p < 0.05$) in both properties with time, although presenting differential reductions. OP sample was the one presenting the highest losses, indicating that the selected carrier agents added preserved to some extent the AOA and ARA. This result can be correlated to the protection of phenolic compounds and vitamin C shown in the solute-containing samples. Comparing these two samples, O-GB showed an enhanced ARA and AOA. This difference could be attributed to the higher VCC in O-GB sample, which suggests a higher stabilizing capacity of BF towards this compound over CM.

The observed evolution of bioactive compounds during storage seems to be related with the availability of water to participate in degradative reactions or to act as a vehicle that allows the mobility of the different substrates involved, which is also related to the physical state of the amorphous phase of the samples. Even though O-GB and O-GC samples were in a glassy state considering the midpoint value of glass transition, this transition occurs over a temperature range from the onset to the endset (Table 2). In this sense, the onset of the glass transition (below 20ºC in all samples) must be considered instead of the midpoint one in order to ensure the nutritive and functional stability of the powder products, since a small

decrease in the viscosity of the medium could be enough to enhance the rate at which the chemical degradation reactions of the analyzed compounds, such as vitamin C, start. Similar results have been obtained in other fruit samples [35].

A statistical correlation was carried out to explain the relationship between the bioactive compounds quantified with the ARA and AOA. A statistical analysis involving the calculation of the Pearson correlation coefficient between each pair of variables was carried out. The results showed that all bioactive components analyzed (TPC and VCC) in the orange powders presented a high and positive, significant contribution to the ARA and AOA ($0.8370 < r < 0.9281$, $p < 0.05$) measured as free radical scavenging activity ($DPPH^{\cdot}$ assay) and as ferric reducing ability (FRAP assay), respectively. For both bioactive components, the highest correlations were found with FRAP assay ($r = 0.9281$ for TPC and 0.8644 for VCC, while the corresponding values for $DPPH^{\cdot}$ assay were $r = 0.8877$ and 0.8370, respectively).

3.4. Compression test and color analysis

The results obtained from the optical and mechanical tests are shown in Table 4. Optical analysis results before storage showed that significant changes ($p < 0.05$) in the color of the powders were promoted by solute addition, without significant differences ($p > 0.05$) between the nature of carrier agents used. The addition of solutes (mainly GA) caused an increase in L* and h* value when compared with OP. On the contrary, a significant ($p < 0.05$) decrease in C* was observed for the solute-containing systems. This effect can be attributed to the incorporation of these materials to the orange pulp, leading to a dilution of the pigments present in the pulp since GA, FB and CM are clear powders with a slight yellow color. In this sense, O-GB and O-GC samples showed a less pure orange color than the OP sample.

Concerning the maximum force attained during compression of all three powders before storage, results showed no significant differences between solute-containing orange pulp

systems ($p > 0.05$), but they do present a significant higher ($p < 0.05$) F_{max} value compared to OP sample. This can be related to the anti-caking properties of the solutes, which increased the T_g of OP sample, assuring the glassy state of samples, reducing stickiness and collapse phenomena [6]. When the glass transition temperature is reached, amorphous materials change from a solid glassy state to a liquid-like rubbery one increasing the molecular mobility and affecting its physical properties. The samples were then subjected to further analysis in order to evaluate the changes in texture and color after 10 months of storage. The obtained parameters are also shown in Table 4. Regarding their color, there were no significant difference ($p < 0.05$) in C* of any of the samples in the storage conditions tested. On the contrary, all three samples showed L* values considerably lower than the measured at the initial storage time, presenting OP sample the sharpest decrease. O-GC sample was the only one suffering a significant change in h*, although it remained significantly higher than the corresponding one to OP sample. The overall color changes (ΔE_t) were more marked for OP samples than for O-GB and O-GC, indicating the selected carrier agents also preserved to some extent the color of samples during storage.

The compression test showed that there was no significant decrease in the F_{max} of the solute-containing powders after 10 months of storage. The increase in T_g as a consequence of the addition of the solutes, makes them more stable from a mechanical point of view, maintaining the samples as free powders, avoiding collapse and caking problems. On the other hand, and as it may be expected for a rubbery material, OP sample suffered an important diminution in its F_{max} value, which leads to the above-mentioned problems.

3.5. FESEM analysis

The morphological structure of the three samples is shown in the micrographs obtainded by FESEM (Fig. 1). The first one (A), corresponding to OP sample, shows structures with rounded corners, in contrast to images (B) and (C), corresponding to O-GB and O-GC,

respectively, which present sharper particles. This difference could be attributed to the capacity of both carrier agents systems to increase the glass transition temperature (T_g) of the orange pulp powder, generating crystalline particles. Moreover, the crushing process produced larger number of small particles in samples O-GB and O-GC, presenting a more sawdust-like morphology, as a consequence of the greater friability of these two glassy samples in comparison with the rubbery sample OP.

4. Conclusions

The results obtained proved the effectiveness of adding gum arabic in combination with bamboo fiber or cactus cladode mucilage to orange pulp to stabilize its bioactivity and to convert it into a powder with acceptable physicochemical properties. This strategy lowered the hygroscopicity of the powder, being the bamboo fiber the one with the highest lowering capacity. Regarding antioxidant properties, freeze drying favored the extraction of phenolic compounds and, therefore, the antioxidant activity of the samples increased with respect to fresh orange puree. Gum arabic and bamboo fiber or cactus cladode mucilage addition proved to be effective to stabilize and protect total phenolic compounds during processing.

Solute-containing powders showed significant higher T_g and maximum forces during compression test compared to orange pulp powder, showing enhanced physical stability. Carrier agents exerted a dilution effect on the orange natural pigments, making powders clearer. According to FESEM analysis, orange pulp powder showed structures with rounded corners, in contrast to solute-containing samples, which presented sharper particles.

The presence of added solids protected the constitutive bioactive compounds during storage, presenting the solute-containing samples higher antiradical and antioxidant activities than orange pulp powder. Comparing both carrier mixtures, gum arabic in combination with bamboo fiber showed a better performance than gum arabic with cactus cladode mucilage.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Table 1. Moisture content (x_w) and hygroscopicity (H) of orange pulp powder (OP) and orange powders with gum arabic in combination with bamboo fiber (O-GB) or cactus cladode mucilage (O-GC)

Sample	x_w (g/ 100 g)	H (g/ 100 g dry matter)
OP	2.59 (0.02) [a]	30.04 (0.03) [a]
O-GB	2.51 (0.01) [b]	26.14 (0.09) [b]
O-GC	2.50 (0.01) [b]	26.79 (0.03) [c]

Different letters within each column indicate significant differences ($p < 0.05$)

509 **Table 2.** Glass transition temperature (T_g) of orange powder (OP) and orange powder with
510 gum arabic in combination with bamboo fiber (O-GB) or cactus cladode mucilage (O-GC).

Samples	T_g onset	T_g midpoint	T_g endset
OP	4.0 (1.2) [a]	10.6 (0.5) [a]	15.8 (0.4) [a]
O-GB	15 (2) [b]	22.9 (0.4) [b]	29.8 (1.4) [b]
O-GC	16 (2) [b]	24.1 (0.8) [b]	33 (5) [b]

511 Different letters within each column indicate significant differences ($p < 0.05$)
512

513

514

515 **Table 3.** Total phenolic content (TPC), vitamin C (VCC) and antiradical (DPPH˙) and
516 antioxidant (FRAP) properties of fresh orange pulp (O), orange powder (OP) and orange
517 powder with gum arabic in combination with bamboo fiber (O-GB) or cactus cladode
518 mucilage (O-GC) before and after storage (10 months).

Sample	Storage time (months)	TPC (1)	VCC (2)	DPPH· (3)	FRAP (3)
O	0	3.9 (0.6) ^{a}	5.3 (0.5) ^{c}	46.7 (1.6) ^{a}	34.4 (1.5) ^{a}
OP	0	5.9 (0.3) ^{b}	4.0 (0.3) ^{a}	50.1 (1.1) ^{ab}	41.4 (1.1) ^{b}
	10	4.1 (0.2) ^{a}	2.6 (0.3) ^{a}	26.2 (0.6) ^{a}	20.8 (0.7) ^{a}
	Losses (%)	31.2*	34.4*	49.8*	47.7*
O-GB	0	6.2 (0.4) ^{b}	5.2 (0.4) ^{bc}	53.1 (1.6) ^{ab}	41.1 (1.5) ^{b}
	10	5.9 (0.1) ^{b}	4.5 (0.3) ^{b}	40.4 (0.9) ^{b}	34.8 (0.7) ^{b}
	Losses (%)	4.6	13.9	15.3*	23.9*
O-GC	0	6.3 (0.3) ^{b}	4.4 (0.3) ^{ab}	53.4 (6.7) ^{b}	40.3 (2.4) ^{b}
	10	5.7 (0.6) ^{b}	3.8 (0.3) ^{a}	36.3 (1.6) ^{c}	30.2 (0.9) ^{c}
	Losses (%)	10.1	14.1	25.1*	32.0*

519 Different letters within each column indicate significant differences among samples at
520 equal storage time ($p < 0.05$)
521 * Losses with storage are significant ($p < 0.05$)
522 (1) g gallic acid equivalents /kg orange's own solutes
523 (2) g ascorbic acid/kg orange's own solutes
524 (3) mmol Trolox equivalents/kg orange's own solutes
525

526

Table 4. Color analysis and compression test parameters of orange pulp powder (OP) and orange pulp powder with gum arabic in combination with bamboo fiber (O-GB) or cactus cladode mucilage (O-GC)

Sample	Storage time (months)	L*	C*	h*	ΔEt [(1)]	F_{max} (N/g)	ΔF_{max} [(2)] (%)
OP	0	83.2 (0.9) [a]	40.5 (1.4) [a]	81.6 (0.4) [a]	6.0	172 (33) [a]	-44.2 [*]
	10	76.4 (0.8) [a]	41.3 (1.3) [a]	82.1 (0.4) [a]		96 (28) [a]	
O-GB	0	86.7 (0.9) [b]	32.7 (0.7) [b]	84.9 (0.3) [b]	5.3	521 (157) [b]	7.9
	10	80.7 (0.5) [b]	34.1 (0.7) [b]	84.1 (0.4) [b]		562 (24) [b]	
O-GC	0	86.7 (0.9) [b]	32.1 (0.6) [b]	84.9 (0.3) [b]	5.5	422 (181) [b]	9.2
	10	80.6 (0.2) [b]	33.8 (0.6) [b]	83.7 (0.2) [b]		461 (54) [b]	

Different letters within each column indicate significant differences among samples at equal storage time ($p < 0.05$)

[(1)] Total color change undergone with storage

[(2)] Change in F_{max} with storage

[(*)] Changes with storage are significant ($p < 0.05$)

Figure Captions

Figure 1. FESEM micrographs of orange pulp powder (Fig. 1A) and orange pulp powder with gum arabic in combination with bamboo fiber (Fig. 1B) or cactus cladode mucilage (Fig. 1C). Magnification: 3000-fold.

Figure(s)

Fig. 1A

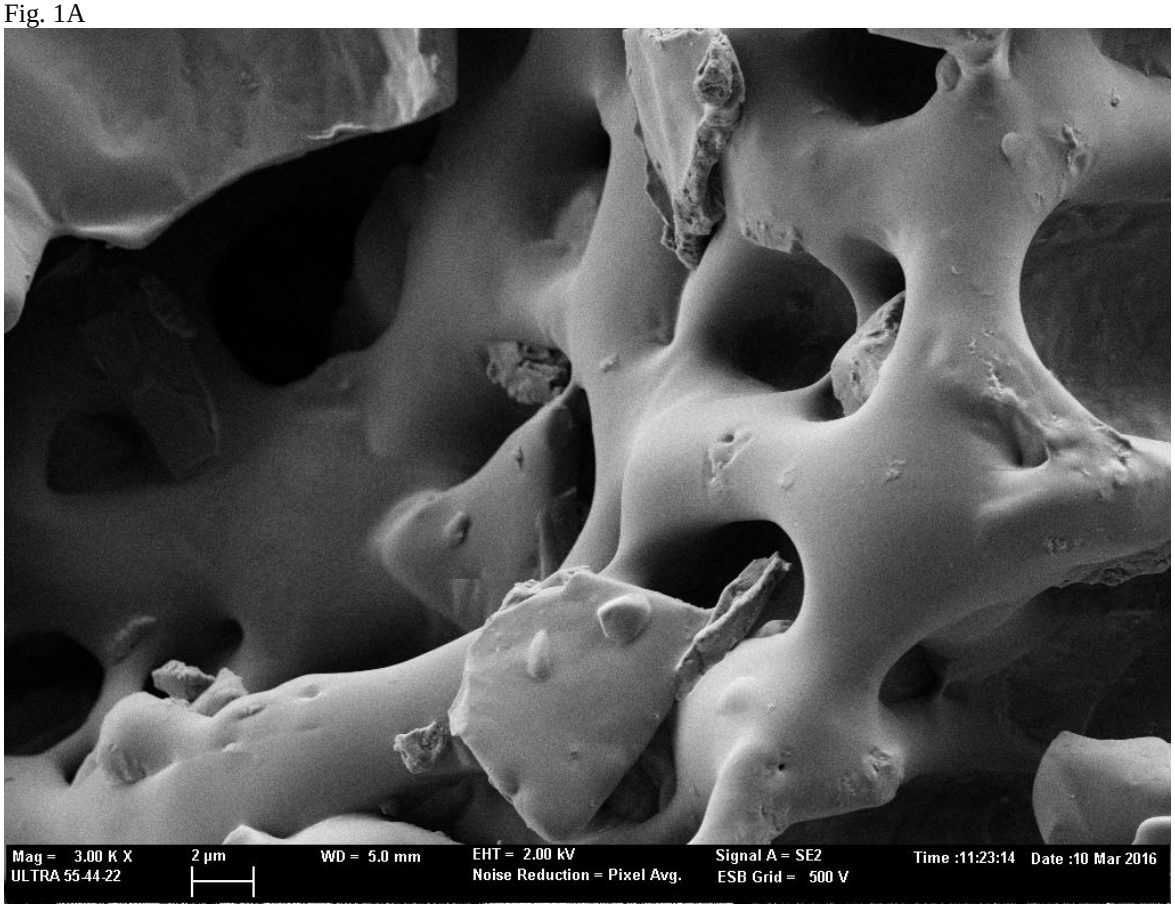


Fig. 1B

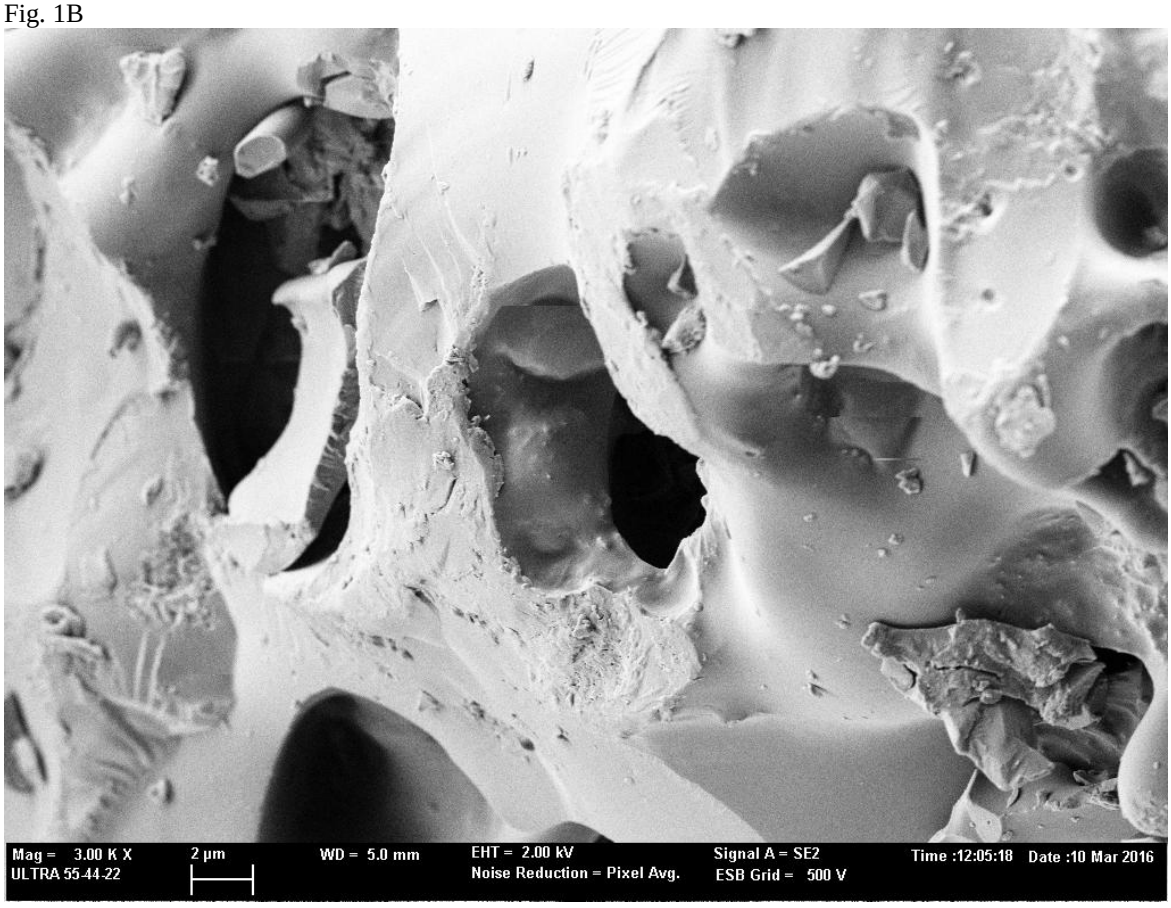
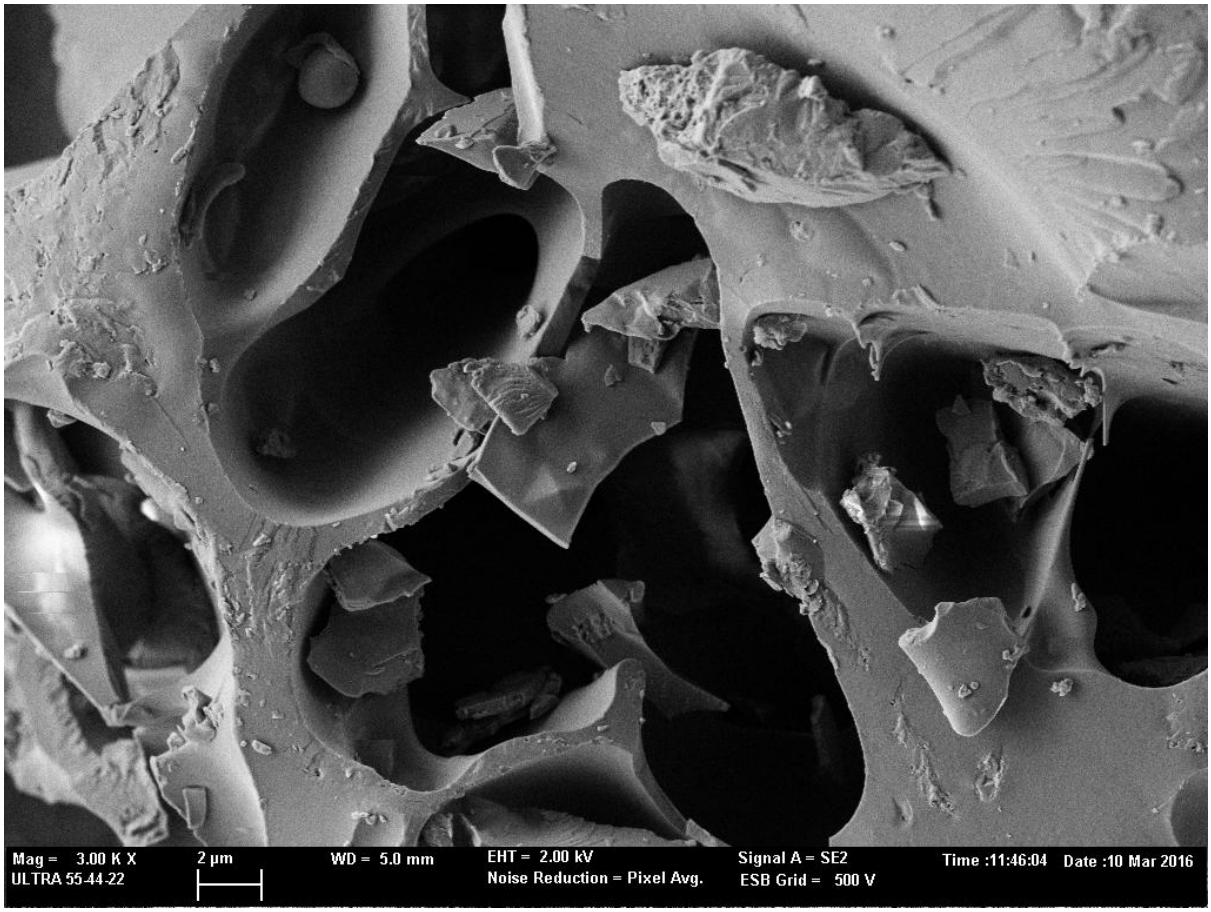


Fig. 1C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